



Quitting smoking and quantification studies of available anti-smoking drugs

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ABSTRACT

Different medications used in successful quitting of smoking are discussed in present article. An attempt was made to develop and validate a novel, simple, cost effective, accurate, and reproducible analytical methods to Varenicline (VRC), Bupropion, nortriptyline and clonidine in bulk and pharmaceutical formulations. Varenicline is a highly selective partial agonist of the nicotinic acetylcholine receptor $\alpha 4\beta 2$ subtype. Varenicline works by preventing nicotine from reaching nicotine receptors in the brain and by stimulating dopamine production both of which make cigarettes less satisfying. Varenicline trials concluded that the drug doubled the chances of successfully quitting compared to unassisted quitting. Bupropion is a second-generation antidepressant agent that is also used in the management of smoking cessation. Analysis of pharmaceutical products covers every aspects of quality control of active pharmaceutical ingredients (API) and finished products. This method was validated according to ICH guidelines. Validated method has proved to be linear, accurate, precise, and robust, it is suitable for pharmacokinetic assays, such as bioavailability and bioequivalence, and is being successfully applied as anti-smoking agent.

Keywords: Varenicline (VRC), Bupropion, Anti-smoking, Liquid Chromatography, Plasma, LC-MS/MS, HPLC

INTRODUCTION

Stopping smoking is always beneficial to health and it is never too late. After the age of 35-40 years, for every year of continued smoking a person loses about 3 months of life expectancy [1]. The evidences are established that the people feel mentally sound once they are free from nicotine addiction. Smoking cessation medications are discussed below:

NICOTINE REPLACEMENT THERAPY (NRT)

Nicotine replacement therapy (NRT) includes nicotine chewing gum, patches, lozenges, mouth spray, inhalator and nasal spray and is usually taken for 8 to 12 weeks. NRT is designed to reduce the motivation to smoke by replacing nicotine from cigarettes. The nicotine replacement medicines are started at the same time as cigarette smoking stops. Cigarettes in comparison to NRT are very much dangerous because the tar present in cigarette smoke along with other toxins causes lung cancer and other smoking-related illnesses. [2] Clinical trials have consistently found that NRT increases the success of quitting smoking by 50-70%. The National Institute for Health and Care Excellence (NICE) recommends that smokers who are committed to quitting should be offered NRT to support their quit attempt and that more heavily addicted smokers may be offered a combination of NRT products if required. Initially smokers feel behavioral changes but the use of NRT aids a lot in successful quitting from smoking.

VARENICLINE (CHAMPIX)

Varenicline is a highly selective partial agonist of the nicotinic acetylcholine receptor $\alpha 4\beta 2$ subtype. Varenicline, a partial agonist, blocks the full-agonist activity of nicotine by competitive binding. It helps in relief of withdrawal and craving symptoms. Because of weakening these symptoms, it endorses smoking cessation. Clinical trials have reliably found that this therapy raises the success of quitting smoking by 50-70% [3]. VRC tartrate (Champix® and Chantix®; Pfizer) has been approved by the USA-FDA as an aid to smoking cessation [4]. The claimed indication

for CHAMPIX (varenicline) 0.5 mg and 1 mg film-coated tablets is smoking cessation in adults. The chemical structure of Varenicline is shown in Figure 1.

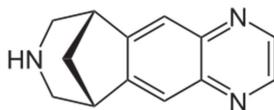


Figure 1: Chemical structure of varenicline

It is highly soluble in water. The climate condition is widely varying worldwide, and consequently, the quality of pharmaceutical product of varenicline (VRC) is greatly affected by the change in the environmental factors. As well, VRC-containing tablets are imported from outside of many countries; consequently there is a major concern about its quality after exposure to transportation, storage, and other conditions. Therefore, quality control for VRC-containing tablets is critically concerned in these countries. In quality control laboratories, analysis of active drug compounds in pharmaceutical formulations is the routine process. Therefore, validation of an analytical method is the key point to propose a newly developed technique to be used in the routine of the quality control. In the literatures we can see different analytical techniques like UPLC, UV-Visible spectrophotometric, HPLC, HPTLC, etc have been used to validate varenicline tartrate [5]. Capillary electrophoresis (CE) is one of preferred techniques in quantitative analysis and particularly considered fast, more selective, economic and precise when comparing with other techniques such as HPLC. Champix® tablets containing the active ingredient varenicline as the tartrate salt have been quantitatively determined in human plasma by liquid chromatography tandem mass spectrometry. The developed reverse phase high performance liquid chromatographic technique was also simple, precise, specific and accurate and the statistical analysis proved that, method is reproducible and selective for the analysis of varenicline tartrate in bulk drug and tablet formulation.

BUPROPION (ZYBAN)

Bupropion, an antidepressant drug having smoking cessation properties were first noticed in the United States of America (US) [6]. Subsequent assessment as an anti-smoking agent, it became licensed as an aid to smoking cessation and is now a renowned first line antismoking agent in both the UK and US. Over the age of 18 years patients Bupropion is licensed and is available in the Sustained Release formulation. The chemical structure of bupropion is shown in Figure 2.

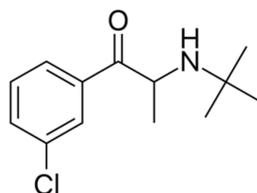


Figure 2: Chemical structure of the bupropion molecule

Bupropion shows its main mechanism of action through dopamine and noradrenalin reuptake inhibition with little serotonergic effect [7].

Electronic Cigarettes

Electronic cigarettes or vapourisers are increasing in popularity as an alternative to smoked tobacco products and proved to be much effective in helping smokers to quit. [8] The harmful effect from smoking is caused primarily through the toxins or tar produced by the burning of tobacco. Electronic cigarettes or vapourisers consist of aerosol of nicotine and some other particles, some flavors, aroma transporters, glycerol and propylene glycol. It contains very less amount of toxicants as compared to cigarettes smoke. One preliminary study found that the concentration of particles in electronic cigarette vapour was about 100-fold lower than from tobacco smoke. If we use placebo or gradually decreasing amount of nicotine with different flavors in electronic cigarettes, it could be very much helpful to control addiction. By contrast, non-tobacco, non-smoked nicotine products, although addictive, are considerably less harmful. It helps people to quit smoking and reduce their cigarette consumption. Interestingly, there is also some proof that people believe EC to be less injurious than medicinal nicotine replacement therapy (NRT). [9] It is not sure that EC products are more or less effective than licensed stop smoking medications, but they are much more popular, thereby giving an opportunity to enlarge the count of smokers discontinuing magnificently.

Other medications to stop smoking

MECAMYLAMINE

Mecamylamine is a drug that is originally used to reduce blood pressure but also found effective in blocking the effect of nicotine. In the preclinical studies, acute pretreatment with nAChR antagonists (mecamylamine) decreased

nicotine self administration by 50% [10]. For an effective smoking cessation high doses of the drug are needed but this carries significant side effects like constipation, drowsiness and hypotension. There is limited research about the effectiveness of the drug in lower doses. The chemical structure of mecamylamine is shown in Figure 3.

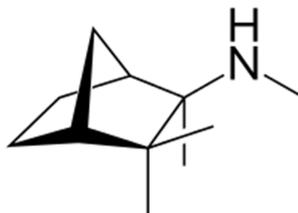


Figure 3: Chemical structure of mecamylamine molecule

CYTISINE

From the last forty years cytisine has been occupied in Eastern Europe market but is not licensed for use in the United Kingdom. Cytisine is a partial agonist with high affinity binding to the $\alpha 4\beta 2$ nicotinic acetylcholine receptor believed to be central to the fulfilling effect of nicotine. [11] There is proof that it is effective but it may be that further research will be required before it can be licensed in the UK. The chemical structure of cytisine is shown in Figure 4.

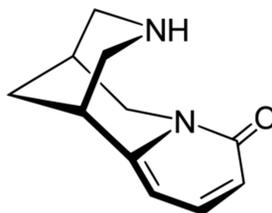


Figure 4: Chemical structure of cytisine molecule

NORTRIPTYLINE

Nortriptyline, an anti-depressant is a successful aid to smoking cessation with a degree of effect similar to that for bupropion and nicotine replacement therapies. [12] Increased risk of suicide ideation is found to be common side effect of nortriptyline. The drug is not presently recommended solely for smoking cessation in the United Kingdom. The chemical structure of Nortriptyline is shown in Figure 5.

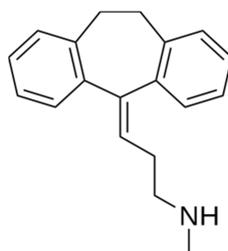


Figure 5: Chemical structure of Nortriptyline molecule

CLONIDINE

Clonidine is one of the choices for the treatment of hypertension but also diminishes the signs of nicotine withdrawal so it is registered as a second-line treatment for smoking cessation in the United States. Glassman and co-workers' reported that clonidine, an α -2- noradrenergic agonist, diminished withdrawal symptoms in a double-blind crossover study on the acute smoking withdrawal syndrome, and recommended that clonidine may have some clinical usefulness in helping heavy smokers to quit [13] The chemical structure of Clonidine is shown in Figure 6.

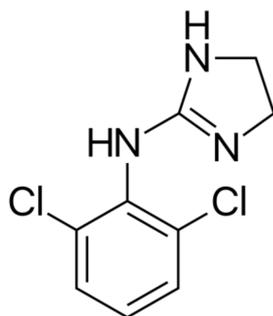


Figure 6: Chemical structure of Clonidine molecule

Quantification studies of varenicline

UV-Visible spectrophotometric study

Kocak et al. 2013[14] developed and validated uv-visible spectrophotometric method for the determination of varenicline in tablets. 0.01 M phosphate buffer with pH 7 was used to prepare standard stock solutions. Uv-visible spectrophotometric determination was done at 319 nm wavelength. The method developed was linear and found in the range within 1-100 $\mu\text{g mL}^{-1}$. ICH guideline was followed to establish the method and the results show that this simple and low cost method is precise, accurate, robust and rugged to be proposed for the routine analysis in quality control laboratories. This method is proposed to be fast and inexpensive in comparison to chromatographic techniques.

High-performance liquid chromatography study

Kadi et al. 2011[15] validated an accurate stability-indicating HPLC method for determination of varenicline in its bulk and tablets forms. Chromatographic separation was achieved on a Zorbax Eclipse XDB-C8 column by a mobile phase consisted of acetonitrile and 50 mM potassium dihydrogen phosphate buffer (10:90 v/v) with apparent pH of 3.5 ± 0.1 and a flow rate of 1.0 ml/min. The wavelength was set at 235 nm. The degradation products were analyzed in comparison with pure drug with significantly dissimilar retention time values. The method was found linear with $r = 0.9998$ and concentration range of 2-14 $\mu\text{g/ml}$. The detection and quantitation limit were found to be 0.38 and 1.11 $\mu\text{g/ml}$, respectively. The intra- and inter-assay precisions were acceptable and the relative standard deviations were within 2%. The accuracy of varenicline was satisfactory with the recovery of $100.10 \pm 1.08\%$. The run time was also very short (~ 6 min). The proposed method established successfully with acceptable accuracy and precisions and the label claim % were $99.65 \pm 0.32\%$. The above results proven that the given method would be very much valuable in quality control and stability studies for varenicline.

Satheesh et al. 2012 [16] found some mysterious impurity during the stability analyses of 0.2% level of varenicline tablets by reversed phase ultra-high pressure liquid chromatography. They developed a simple isocratic method to isolate the unknown impurity with 20 min run time. They used some latest spectroscopic techniques to identify the impurity as 4,6,7,8,9,10 - hexahydro-1 H-6,10- methanopyrazino[2,3 -h][3]benzazepine-2,3-dione. The structure of this impurity was also established clearly, prepared by isolation and co-injected into UHPLC to approve the retention time.

Channabasavaraj et al. 2011 [17] developed and validated a reverse phase high performance liquid chromatographic method for the estimation of Varenicline tartrate in bulk and tablet using UV detector. C-18 column was used as gradient chromatography with a mobile phase composed by Methanol: Potassium dihydrogen orthophosphate buffer pH 3 (50:50, v/v), at flow rate of 0.6 ml / min using UV detection at 237 nm. The retention time for Varenicline tartrate was found to be 2.966 min. Linearity of the method was found to be 10 to 50 $\mu\text{g/ml}$, with the regression coefficient of 0.9999. This method was validated according to ICH guidelines. The intra-day and inter day percentage relative standard deviation (RSD) was found 0.327 and 0.147 respectively. The proposed method was effectively applied for the quantitative determination of Varenicline tartrate in tablet formulations.

Mass spectrometry study

Al-Haj et al. 2013 [18] developed a method for quantitative determination of varenicline in human plasma grounded on liquid chromatography coupled to tandem mass spectrometry. They first extracted varenicline and the internal standard 25.0 ng/mL of clarithromycin from human plasma, using methyl tertiary butyl ether as the organic solvent. C8 column with isocratic elution using a mixture of acetonitrile: 0.001 M ammonium acetate (70:30%, v/v) were used. Analyses were run at pH 4.0. The concentration range of 0.1–10.0 ng/mL were used to validate the process by investigating specificity, sensitivity, linearity, precision, accuracy, recovery, matrix effect and stability according to

United State Food and Drug Administration guideline. This method magnificently used to estimate bioequivalence of two commercial products of 1 mg varenicline.

Piestansky et al. 2014 [19] developed advanced analytical method, based on two-dimensional column coupled CE (ITP-CZE) hyphenated with tandem mass spectrometry (MS/MS, here triple quadrupole, QqQ). Capillary isotachopheresis (ITP) coupled on-line with capillary zone electrophoresis (CZE) used in hydrodynamically closed separation system. In this methodology CZE-ESI combination was used to intensify the MS detection signal and reproducibility of the analyte peak area. The enhanced ITP-CZE-ESI-QqQ method effectively estimated for parameters like LOD, LOQ, linearity, precision, recovery/accuracy. This served as currently the most effective routine clinical method for varenicline.

Dobrinias et al. 2011 [20] developed and validated a sensitive and precise UPLC-tandem MS method for the quantification of nicotine, its metabolites cotinine and trans-3'-hydroxycotinine and varenicline in human plasma. Solid phase extraction technique used for sample preparation. Nicotine-d4, cotinine-d3, trans-3'-hydroxycotinine-d3 and CP-533,633, a structural analog of varenicline used as internal standards. Separations were done on a hydrophilic interaction liquid chromatography column, 2.1 × 100 mm, 1.7 μm. Quadrupole mass spectrometer was used as detector and quantification was accomplished using multiple reaction monitoring. Matrix effects were quantitatively evaluated with success, with coefficients of variation inferior to 8%. The concentration range was 2–500 ng/mL, 2–1000 ng/mL and 1–500 ng/mL used for nicotine, trans-3'-hydroxycotinine and varenicline respectively. Trueness, repeatability and intermediate precision were established to be acceptable, as well as stability in plasma. The technique was effectively used to quantify nicotine, its metabolites and varenicline in more than 400 plasma samples from participants in a clinical study on smoking cessation.

Coe et al. 2009 [21] studied the therapies addressing nicotine addiction associated with the receptor interactions. It deliberates a medicine that decreases nicotine craving and withdrawal symptoms, with pleasingly control on neurochemical strengthening produced by smoking. Varenicline is the first medicine for nicotinic acetylcholine receptors (nAChRs), other than nicotine as replacement therapy (NRT), and is approved as an aid to smoking cessation by both the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) in 2006. It explains that varenicline has great affinity only for the α4β2 neuronal nicotinic receptor subtype in rat and human cortex. Varenicline did not bind with significant affinity to various other neurotransmitter receptors and transporters, enzymes, modulatory binding sites, and ion channels in membranes. Because of its selectivity towards α4β2 neuronal nicotinic receptor, it shows insignificant side effects.

Liquid chromatographic study

Ramzia et al. 2016 [22] developed two simple, sensitive, rapid, and stability-indicating liquid chromatographic (LC) methods for the determination of varenicline tartrate. They included the identification of varenicline (VRC) in the presence of its oxidative degradates and related impurity *N*-formyl varenicline. The method used was a LC with diode array detection (DAD) at 235 nm. Isocratic elution of varenicline was engaged using a mobile phase containing of buffer mixture (1.2% potassium dihydrogen phosphate and 0.08% octane sulphonic acid): acetonitrile (86:14, v/v), pH (5.0). Fluorimetric detection technique was developed in the second method that was created on precolumn derivatization of varenicline using 7-chloro-4-nitrobenzo-2-oxa-1, 3-diazole (NBD-Cl). The fluorescence detector was used and the excitation was done at 539 nm for emission. Isocratic elution was done with a mobile phase consisting of methanol-distilled water (70:30, v/v). Linearity, accuracy and precision were found to be satisfactory over the concentration ranges of 0.5–20.0 μg mL⁻¹ and 0.2–20.0 μg mL⁻¹ with the first and the second method, respectively. The optimized methods were authenticated and verified to be specific, simple, and accurate for the quality control of the drug in its pharmaceutical dosage.

Crystallography study

Murphy et al. 2010 [23] checked the thermodynamic stability association between the three known crystal forms of Varenicline L-tartrate. They utilized crystallographic, spectroscopic, and thermal analysis data technique for this study. Form B, out of two forms was identified to be the stable form at 0 K based on its considered true density, hydrogen bonding in the crystal lattice, and application of the Infra-red rule. Form A found to be higher melting point and higher solubility at room temperature. Submission of the eutectic-melting method aided accurate determination of the transition temperature at 63 °C. A phase boundary was recognized, with the monohydrate being the thermodynamically stable form above critical water activity values of 0.85 and 0.94 for Forms A and B, respectively. These results provide a better understanding of the form stability as it relates to normal manufacturing and storage conditions for the active pharmaceutical ingredient and drug product.

Capillary zone electrophoresis study

Alhazmi *et al.* 2013 [24] developed a fast, sensitive and selective method for the finding and quantification of varenicline in tablet dosage form by using capillary zone electrophoresis separation with DAD detector. The used capillary was uncoated standard bare fused-silica with 100 μm I.D, 40 cm total length and 31.5 cm effective length. The electrophoretic separations were held out in 50 mM sodium phosphate buffer (pH 2.5) under normal mode (30 kV). Samples were filtered through a 0.22 μm CME membrane filter and then injected hydrodynamically at a pressure of 50 mbar for 10 s. The calibration curve of VRC was linear, $r = 0.998$ over the concentration range 1–16 $\mu\text{g/ml}$. The LOD and LOQ values were 0.2 and 0.6 $\mu\text{g/ml}$, respectively. The method proved to be suitable for quality control studies. In this method as the run time of 6 min made it possible to analyze more samples per day and the method is found to be economic due to reduced consumption of references pharmaceuticals materials, solvents and sample.

Microwell-plate spectrophotometric study

Alshehri *et al.* 2014 [25] studied the spectrophotometric investigation for the condensation reaction between varenicline and cyclohexa-3,5-diene-1,2-dione (CHDD) that shows the maximum absorption peak (λ_{max}) at 540 nm. This color-developing reaction was employed in the development of microwell plate assay for varenicline. In this assay, the reaction was carried out in 96-microwell plate and the absorbance of the colored-product was measured by microwell plate absorbance reader. Absorbance with varenicline concentration was found in the range of 5 – 100 $\mu\text{g/mL}$ with good correlation coefficient (0.9986). The detection limits was 2.29 and quantification value was 6.95 $\mu\text{g/mL}$. Values of relative standard deviations (RSD) did not exceed 2%. No interference was observed from the excipients that are present in VRC-containing tablets. The label claim percentages were $98.80 \pm 1.30\%$. The proposed assay is practical and valuable in terms of its routine application in determination of VRC in its bulk and tablets in pharmaceutical quality control laboratories.

Quantification studies of bupropion**High-performance liquid chromatography study**

Thakar *et al.* 2013 [26] developed and validated a novel, rapid, sensitive, accurate and specific HPLC assay with UV-Visible detection (250 nm) for the determination of bupropion hydrochloride in rat plasma. As internal standard, Phenacetin was used. A single step protein precipitation extraction procedure was used to precipitate plasma proteins. Chromatographic separation was achieved with a combination of acetonitrile and 0.01 M potassium dihydrogen phosphate in the gradient mode on a C18 (250 mm \times 4.6 mm, 5 μm) analytical column. Mean recovery of bupropion hydrochloride from rat plasma was around 55 % for 2.5-50 $\mu\text{g/ml}$ concentrations. The assay displayed good linear relationship with an r^2 of 0.9999. Lower limit of Quantification limit was 1.84 $\mu\text{g/ml}$ of bupropion hydrochloride and accuracy and precision were over the concentration range 2.5-50 $\mu\text{g/ml}$. The assay can be used successfully to pharmacokinetic studies.

Phani *et al.* 2015 [27] developed and validated a simple, sensitive, precise, accurate and cost effective reverse phase HPLC method for the simultaneous estimation of bupropion HCl and naltrexone HCl from a single formulation. The two drugs were eluted under isocratic mode using a 250 x 4.6 mm i.d, 0.45 μm Phenomenex Chromosil C18 column. The mobile phase was composed of a mixture of methanol, acetonitrile and water in the ratio of 60:20:20 v/v (pH adjusted to 4.8) at a flow rate of 1.0mL/min. For both the drugs the limit of detection and quantification were found to be 0.5 $\mu\text{g/mL}$ and 1.7 $\mu\text{g/mL}$ respectively. The label claim of the combined dosage form was found to be good with the experimental assay amounts; 99.5% and 99.7% for bupropion HCl and naltrexone HCl, respectively. This method can be positively applied for the estimation of bupropion HCl and naltrexone HCl combination for routine quality control analysis.

Srikalyani *et al.* 2013 [28] developed a simple, specific and accurate RP-HPLC method for the simultaneous analysis of Naltrexone hydrochloride and Bupropion hydrochloride in bulk and dosage forms. A Phenomenex C18 column (250 x 4.6mm; 5 μm) with mobile phase containing 0.05% v/v triethylamine (pH6.5): acetonitrile (45:55% v/v) was used at isocratic mode and eluents were monitored at 215 nm. The retention times of NTX and BUP were 3.4 min and 12.7 min respectively and showed a good linearity in the concentration range of 40-200 $\mu\text{g/mL}$ of NTX and 10- 50 $\mu\text{g/mL}$ of BUP with a correlation coefficient of 0.999 and 0.998 respectively. The average recoveries were found to be 98.60% and 98.90% respectively for NTX and BUP. The method was validated as per ICH guidelines and successfully applied to the simultaneous assessment of NTX and BUP in bulk and dosage forms.

Masters *et al.* 2016 [29] developed novel stereoselective liquid chromatography–tandem mass spectrometry (LC–MS/MS) method to separate and quantify enantiomers of bupropion, 4-hydroxybupropion, and erythro- and threo-dihydrobupropion. Liquid-liquid extraction was implemented to extract all analytes from 50 μL human plasma. Acetaminophen was used as an internal standard. Less than 9.8% variability for all analytes was found for over 48 h at ambient temperature.

Katarzyna *et al.* 2005 [30] developed a specific and reproducible HPLC assay to simultaneously quantify bupropion and its major metabolites hydroxybupropion, threohydrobupropion and erythrohydrobupropion in human plasma. The analysis was done on an aqua C18 HPLC column, with a mobile phase consisting of 45:55 of methanol: 0.05 M phosphate buffer (pH 5.5) and UV detection at 214 and 254 nm (bupropion, internal standard timolol maleate). The assay showed a linear response for bupropion (2.5–250 ng/mL), threohydrobupropion (5–250 ng/mL), erythrohydrobupropion (10–250 ng/mL) and hydroxybupropion (10–1000 ng/mL). Extraction recovery was found to be reproducible and greater than 55% for each analyte. This assay is more sensitive than currently published methods using HPLC with UV detection for the simultaneous quantitation of bupropion and metabolites.

Zhang *et al.* 2003 [31] developed and validated a sensitive HPLC method for the quantitative determination of bupropion in dog plasma. The compound and the internal standard hydroxyl ethyl fludiazepam were extracted from the plasma samples by liquid–liquid extraction. The extracts were analyzed by a reversed-phase HPLC with 50 mmol/l phosphate buffer (pH 5.5)–methanol (45:55, v/v) as the eluent. The assay was specific for bupropion. The calibration curves were linear in the range between 1 and 750 ng/ml. The C_{max} of sustained-release tablet was significantly lower than that of the RT and the T_{max} was significantly longer than that of the RT ($P < 0.05$). The relative bioavailability was $99.1 \pm 1.51\%$, the results of ANOVA and two one sided tests indicated that the new ST exhibited good sustained release properties and was bioequivalent to the RT.

Mass spectrometry study

Yeniceli *et al.* 2011 [32] developed and validated a specific and highly sensitive LC–ESI-MS method for the direct determination of bupropion and its main metabolite hydroxybupropion in rat plasma and brain microdialysate. Plasma samples were analyzed after a simple, one-step protein precipitation clean-up with trichloroacetic acid enabling direct injection of the samples into the LC–ESI-MS system. Signals of the compounds were monitored under the multiple reaction monitoring modes of the LC–ESI-MS ion trap for quantification. The precursor to product ion transitions of m/z 240–184 and m/z 256–238 were used to measure BUP and HBUP, respectively. The intra- and inter-day assay variability was less than 15% for both analytes. This LC–ESI-MS method provided simple sampling, rapid clean-up and short analysis time (<9 min), applicable to the routine therapeutic monitoring and pharmacokinetic studies of BUP and HBUP.

Wang *et al.* 2012 [33] developed and validated a liquid chromatography in tandem with electro-spray ionization mass spectrometry method for the quantitative determination of bupropion and its major metabolites (hydroxybupropion, threo- and erythrohydrobupropion) in human umbilical cord plasma and placental tissue. Chromatographic separation of drug and metabolites was attained by using a waters symmetry C18 column, with an isocratic elution of 31% methanol and 69% formic acid (0.04%, v/v) aqueous solution at a flow rate of 1.0 mL/min. Detection was carried out by mass spectrometry using positive electro-spray ionization mode, and the compounds were monitored using multiple reactions monitoring method. Calibration curves were linear ($r^2 > 0.99$) in the tested ranges. The lower limit of quantification of analytes in umbilical cord plasma samples is <0.72 ng/mL and 0.92 ng/g in placental tissue samples. The extraction recovery of the four analytes extended between 89% and 96% in umbilical cord plasma, and 64% and 80% in placental tissue.

Quantification studies of nortriptyline

High-performance liquid chromatography study

Kumar *et al.* 2015 [34] developed a simple, precise, rapid, specific and accurate stability indicating reverse phase HPLC method for simultaneous estimation of Gabapentin (GPT) and Nortriptyline (NTL) in pharmaceutical tablet dosage form. The detection was carried out at 254 nm. The retention times of GPT and NTL were found to be 2.66 and 3.58 mins respectively with a run time of 6 mins, theoretical levels for GPT and NTL were 8734 and 8648 respectively, with a resolution of 6.56. This demonstrates that the developed method is simple, precise, rapid, selective, accurate and reproducible for simultaneous estimation of GPT and NTL tablet dosage form.

High performance liquid chromatography is the most powerful and versatile instrumental technique used for the detection and quantitation of chemical components in the complex matrices frequently encountered in pharmaceutical analysis [35]. Liquid chromatography combined with mass spectrometry (LC-MS) is considered as one of the most important techniques of the last decade of 20th century [36, 37]. Several HPLC and LC-MS methods have been reported for the determination of amitriptyline and nortriptyline biological fluids.

Quantification studies of clonidine

Devanaboyina *et al.* 2012. [38] developed and validated new simple and accurate isocratic reverse phase high performance liquid chromatography assay for the assessment of clonidine in tablet formulation. The separation was achieved by using C-18 column (250x4.6mm, 5 μ m in particle size) at ambient temperature coupled with a guard

column of silica in mobile phase Methanol : Acetonitrile : water with the pH value adjusted to 5.8 . The projected method was found to be accurate, repeatability and consistent.

Saravanan et al. 2104. [39] developed an isocratic rp HPLC method for the quantitative determination of Hydrochlorothiazide and Clonidine HCl in combined dosage form. A thermo agilent zorebax sb C18 column with mobile phase containing methanol: ortho phosphate buffer in the ratio of 40:60 % was used. The flow rate was 1.0 ml/min, column temperature was 30 °C and effluents were monitored at 217 nm. The proposed method was validated with respect to linearity, accuracy, precision, specificity, and robustness. The method was applied to the simultaneous estimation of Hydrochlorothiazide and Clonidine HCl in combined dosage form due to its simplicity, rapidness and high precision.

CONCLUSION

In the present literature different therapy and medications have been discussed that help to quit smoking. Method validation of established drugs like varenicline, bupropion, nortriptyline and clonidine are also been discussed. These methods have proved to be linear, accurate, precise, and robust, it is suitable for pharmacokinetic assays, such as bioavailability and bioequivalence, and is being successfully applied as anti-smoking agent. Hence, these methods can be readily incorporated into the routine testing of these drugs.

REFERENCES

- [1] R Doll; R Peto. *Brit. Med. J.*, **1994**, 309, 901-11.
- [2] R Richmond; N Zwar. *Drug & Alcohol Rev.*, **2003**, 22(2), 203-20.
- [3] LF Stead; R Perera; C Bullen; D Mant; JH Boyce; K Cahill; T Lancaster. *The Cochrane Library*, Sep. **2012**.
- [4] L Zieler-Brown; J Kyle. *Ann. Pharmacother.*, **2007**, 41, 95-99.
- [5] B Satheesh; S Kumarpulluru; V Raghavan; D Saravanan. *A. Chrom.*, **2010**, 22(2), 207-18.
- [6] EK Tong; TP Carmody; JA Simo. *Compreh. Ther.*, **2006**, 32, 26-33.
- [7] C Warner; M Shoaib. *Addic. Biol.*, **2005**, 10, 219-31.
- [8] H McRobbie; C Bullen; P Hajek. *The Cochrane Libr.*, **2012**.
- [9] A McNeill; LS Brose; R Calder; SC Hitchman. PHE publications gateway. **2015**, 1-113.
- [10] C Cohen; OE Bergis; F Galli; AW Lohead; S Jegham; B Biton. *J. Pharmacol. Exp Ther.*, **2003**, 306, 407-20.
- [11] W Zatonski; M Cedzynska; P Tutka and R West. *Tob Control.*, **2006**, 15(6), 481-84.
- [12] JR Hughes; LF Stead; T Lancaster. *Nicotine Tob Res.*, **2005**, 7(4), 491-99.
- [13] W Hao; Y Derson. *Brit. J. Addic.*, **1988**, 83, 1221-26.
- [14] E Koçak; M Celebier; S Altinöz, *Asn. J. Chem.*, **2013**, 25(4), 1845-48.
- [15] AA Kadi; MS Mohamed; MG Kassem; IA Darwish. *Chem. Centr. J.*, **2011**, 5, 30.
- [16] B Satheesh; K Kondayampettai; G Sree ; S. Dhandayutham, *Sci. Pharm.*, **2012**, 80(2), 329-36.
- [17] KP Channabasavaraj; SJagadish; SHM Modiya. *Int. J. Pharm. Sci.*, **2011**, 3(2), 5961.
- [18] AH Ayoub; A Mahmoud; T Arafat; M K Hourani. *J. Chromat. B.*, **2013**, 931, 134-39.
- [19] J Piastansky; K Marakova; L Veizerova; J Galba; P Mikus; *Analy. Chimic. Acta.*, **2014**, 826, 84-93.
- [20] M Dobrinias; E Choong; M Noetzli; J Cornuz; N Ansermot. *B. Chin. J. Chromat. B.*, **2011**, 30(15), 3574-82.
- [21] JW Coe; H Rollema; TO Brian. *Annual Reports in Medicinal Chemistry.*, **2009**, 44, 71-101.
- [22] IE Ramzia; FA Nisreen; A Marwa; EW Mohamed. *Talanta.*, **2016**, 146, 83-92.
- [23] BJ Murphy; J Huang; MJ Casteel; A Cobani; JF Krzyzaniak. *J. Pharm. Sci.*, **2010**, 99 (6), 2766-76.
- [24] HA Alhazmi; HA Makeen; S El Deeb. *Dig. J. Nanomat. & Biostruct.*, **2013**, 8(1), 295-301.
- [25] MM Alshehri; IA Darwish; MG Kassem; HM Maher; NZ Alzoman. *Dig. J. Nanomat. & Biostruct.*, **2014**, 9(2), 641-51.
- [26] DS Thakar; A. Varghese. *Int. J. Pharm. Biosci. Technol.*, **2013**, 1(1), 20-26.
- [27] RS Phani; D Chaitanya ; B Prasanthi. *Inter. J. Pharm. Scien. and Res.*, **2015**, 6(7), 2982-90.
- [28] V Srikalyani; M Tejaswi; P Srividya; NN Buchi. *J. Chem. and Pharm. Res.*, **2013**, 5(11), 429-35.
- [29] AR Masters; M McCoy; DR Jones; Z Desta. *J. Chromat. B.*, **2016**, 1015, 201-08.
- [30] K Katarzyna; AS Loboz; JR Gross; AJ McLachlan. *J. Chromatography. B.*, **2005**, 823(2), 115-21.
- [31] D Zhang; B Yuan; M Qiao; L Famei. *J. Pharm. and Biomed. Analy.*, **2003**, 33(2), 287-93.
- [32] D Yeniceci; E Şener; OT Korkmaz; D Doğrukol-Ak; N Tuncel. *Talanta.*, **2011**, 84(1), 19-26.
- [33] X Wang; DI Vernikovskaya; DR Abdelrahman; GDV Hankins; MS Ahmed; TN Nanovskaya. *J. Pharm. and Biomed. Analy.*, **2012**, 70, 320-29.
- [34] MN Kumar; DS Malakondaiah; GU Sree; A Ajitha; VUM Rao; *Intern. J. Pharm. Res. & Analy.*, **2015**, 5(1),13-17.
- [35] R Martino; MV Malet-Martino; Gilard and S Balayssac. *Analyt. Bioan. Chem.*, **2010**, 398, 77.
- [36] II Salim; M Al-khatib; N Najib. *J. Pharm. Biomed. Anal.*, **2011**, 56, 983.
- [37] L Chytil; B Strauch; J Cvacka; VJW Maresova; R Holaj; OJ Slanar. *B. Chromatogr.*, **2010**, 878, 3167.

[38] N Devanaboyina; BC Kumar; B Vijay; MA Bhanu; V Gayathri. *Jamonline.*, 2012, 2(1),93-102.

[39] G Saravanan; M Padmaja; A Lakshmi; Gowthami; I Krishnanjaneyulu, *J. Chem. and Pharm. Scien.*, 2014, 7(3), 224-28.