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Journal of Chemical and Pharmaceutical Research, 2016, 8(6):415-422

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Determination of Genotoxic impurities in a nootropic drug, Rivastigmine tartrate by GCMS

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ABSTRACT

A highly sensitive rapid Gas Chromatograph with mass spectrometer (GCMS) method has been developed and validated for the determination of genotoxic impurities, i.e Methyl N-Ethyl-N-Methylcarbamate, Ethyl N-Ethyl-N-Methylcarbamate and N-Ethyl-N-Methylcarbamoyl chloride contents in Rivastigmine tartrate drug substance. The lower level of detection was achieved on Capillary GC column (DB-35, Fused silica capillary column; 30 m length; 0.32 mm internal diameter, coated with 35% phenyl and 65% dimethylpolysiloxane stationary phase of 0.5 µm film thickness with Electron Impact ionization (EI) in Selective Ion Monitoring (SIM) mode. The developed method was validated for specificity, linearity, accuracy and precision. The detection limits of Methyl N-Ethyl-N-Methylcarbamate, Ethyl N-Ethyl-N-Methylcarbamate and N-Ethyl-N-Methylcarbamoyl chloride obtained were 2.0 µg/g each. The method was found to be linear in the range between 6 µg/g and 120 µg/g with correlation coefficient 0.9993, 0.9989 and 0.9988 respectively. The average recovery range obtained for these three impurities was between 97.3 % and 108.7%.

Keywords: Rivastigmine tartrate, N-Ethyl-N-Methylcarbamoyl chloride, Methyl N-Ethyl-N-Methylcarbamate, Ethyl N-Ethyl-N-Methylcarbamate, GCMS

INTRODUCTION

Alzheimer's disease (AD) is a mutifactorial disorder with an unclear etiology. Some of the potential causative or contributing factors implicated in AD include genetic and epigenetic factors [1-2], diet, physical activity [3] and other gene-environment interactions [4]. Psychological attributes of the individual, such as a subjective sense of a purposeful life, may also correlate with risk of developing AD. One important therapeutic goal in AD treatment is to restrict the progression and/or delay the onset of the disease. Currently, the cholinesterase inhibitors (ChEI) tacrine, donepezil, rivastigmine, galantamine and a partial NMDA receptor antagonist memantine are the only drugs approved by the FDA for treatment of AD [5-6]. Rivastigmine treatment enhances neuronal sAPP and shifts APP processing toward the α-secretase pathway in degenerating neuronal cultures, which mirrors the trend of synaptic proteins, and metabolic activity [7]. Rivastigmine is a parasympathomimeticor cholinergic agent for the treatment of mild to moderate dementia of the Alzheimer's type and dementia due to Parkinson's disease. Rivastigmine is a semi-synthetic derivative of physostigmine [8]. Rivastigmine Tartrate is chemically known as (*S*)-*N*-ethyl-*N*-methyl-3-[1-(dimethylamino)-ethyl]phenylcarbamate hydrogen (2*R*,3*R*)-tartrate, molecular formula is $C_{14}H_{22}N_2O_2.C_4H_6O_6$ and molecular weight is 400.42, the chemical structure of Rivastigmine Tartrate is shown in Figure 1.

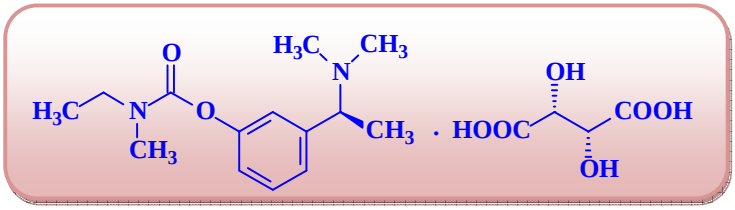


Figure 1. Chemical structure of Rivastigmine Tartrate

The following impurities Methyl N-Ethyl-N-Methylcarbamate, Ethyl N-Ethyl-N-Methylcarbamate and N-Ethyl-N-Methylcarbamoyl chloride are likely present in Rivastigmine tartrate drug substance. In these, N-Ethyl-N-Methylcarbamoyl chloride is used as a raw material for the preparation of Rivastigmine tartrate. The other two are possible impurities, due to use of methanol and ethanol in subsequent steps of Rivastigmine process, these two solvents may react with N-Ethyl-N-Methylcarbamoyl chloride raw material and gives corresponding alkyl carbamate impurities. Based on literature and evaluation by Derek software, these three compounds are found to be mutagenic and carcinogenic. Hence, these genotoxic impurities are limited to a daily dose of 1.5µg/day as per ICH guidelines from the European medical agency [9-10]. Hence, in order to meet the regulatory agencies requirements, it is essential to develop a sensitive analytical method. Hence, a gas chromatograph with mass spectrophotometer was chosen which can detect low level determinations for the quantification of Methyl N-Ethyl-N-Methylcarbamate, Ethyl N-Ethyl-N-Methylcarbamate and N-Ethyl-N-Methylcarbamoyl chloride. The chemical structures of these three impurities are shown in Figure 2a, Figure 2b & Figure 2c respectively.

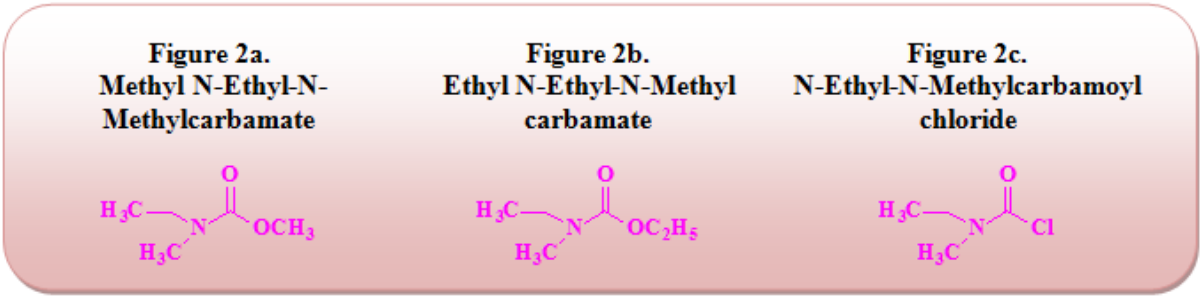


Figure 2. Chemical structures of impurities

By considering a maximum daily dose of Rivastigmine Tartrate drug substance and TTC approach for potential genotoxic impurities, the limit Together NMT 78 µg/g for three impurities chosen as specification level for this research work. The developed method was validated for specificity, sensitivity (Limit of Detection and Limit of Quantitation), linearity, precision (system precision, method precision and intermediate precision) and accuracy in accordance with ICH Q2 (R1) [11].

EXPERIMENTAL SECTION

Chemicals, reagents and samples

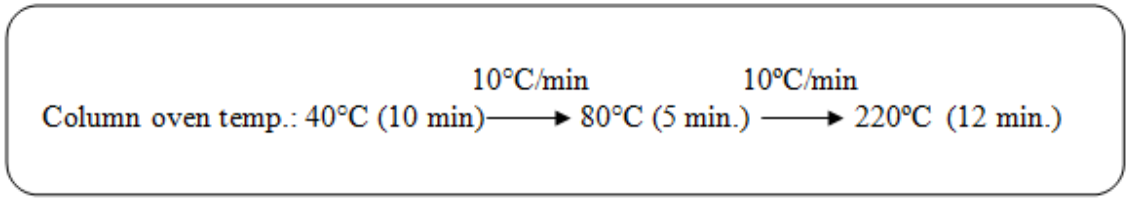
The investigated Rivastigmine tartrate drug substance and three impurities Methyl N-Ethyl-N-Methylcarbamate , Ethyl N-Ethyl-N-Methylcarbamate and N-Ethyl-N-Methylcarbamoyl chloride were gifted from APL Research Centre laboratories (A division of Aurobindo Pharma Ltd., Hyderabad.). Analytical reagent (AR grade) Ethyl benzene and Methylene chloride were procured from Fluka Germany.

Equipment

The gas chromatograph system with mass spectrometer, Shimadzu GCMS-QP2010 equipped with Shimadzu AOC-5000 head space sampler (Make: Shimadzu Corporation, Kyoto, Japan) was used. The data handling system, GCMS solution, version 2.53.00 SU1 was used to monitor the output signals and for processing.

Chromatographic conditions

The analysis was carried out on Capillary GC column (DB-35, Fused silica capillary column; 30 m length; 0.32 mm internal diameter, coated with 35% phenyl and 65% dimethylpolysiloxane stationary phase of 0.5 µm film thickness) (Make: J & W Scientific, Santa Clara, CA, USA). Helium gas was used as carrier gas, maintaining column pressure at 10kPa in a split mode with ratio of 1:2. The temperature of the capillary injector was set as 180°C and column oven temperature was programmed as given below:



The mass parameters were set as follows.

Ion source temperature 250°C, Interface temperature 200°C; Threshold 200 and Detector voltage should be relative to the tuning results.

Group & Events					Channel (*m/z*)			
Name	**Start time (min)**	**End time (min)**	**Acq. Mode**	**Event time (sec)**	**A**	**B**	**C**	**D**
Ethyl benzene	12.10	13.00	SIM	0.20	65	77	91	106
Methyl N-Ethyl-N-Methylcarbamate	14.50	15.20	SIM	0.20	58	86	102	117
Ethyl N-Ethyl-N-Methylcarbamate	17.10	17.80	SIM	0.20	44	58	116	131
N-Ethyl-N-Methylcarbamoyl chloride	18.20	19.10	SIM	0.20	58	86	106	121

Preparation of solutions

Internal standard solution:

Accurately weigh and transfer about 0.07 g of Ethyl benzene into a 10 ml clean, dry volumetric flask containing about 5 ml of Methylene chloride, mix and make up to volume with Methylene chloride. Dilute 0.5 ml of this solution to 50 ml with Methylene chloride. Further dilute 0.5 ml of this solution to 250 ml with Methylene chloride (1.4µg/ml)

Blank solution:

Filter about 5 ml of Internal standard solution through PTFE filter of 0.45 µm pore size, initially discard the about 2 ml of the solution and collect the filtrate for injection.

Standard solution:

Accurately weigh and transfer each about 0.0312 g each of Methyl N-Ethyl-N-Methylcarbamate, Ethyl N-Ethyl-N-Methylcarbamate and N-Ethyl-N-Methylcarbamoyl chloride into a 10 ml clean, dry volumetric flask containing about 5 ml of internal standard solution, mix and make up to volume with internal standard solution. Dilute 0.5 ml of this solution to 50 ml with internal standard solution. Further dilute 0.5 ml of this solution 50 ml with internal standard solution. Filter about 5 ml of this solution through PTFE filter of 0.45 µm pore size, initially discard the about 2 ml of the solution and collect the filtrate for injection. (3.12µg/ml)

Sample solution:

Accurately weigh and transfer about 0.04 g of sample into a 10 ml clean, dry volumetric flask containing about 5 ml of internal standard solution and shake vigorously for about 5 min. Make up to volume with internal standard solution. Filter this solution through PTFE filter of 0.45 µm pore size, initially discard the about 2 ml of the solution and collect the filtrate for injection. (40000µg/ml)

RESULTS AND DISCUSSION

Method Validation

Specificity

As per ICH guidelines, specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The specificity of the developed GCMS method was verified in presence of

residual solvents like Methanol, Acetone, Acetonitrile, Toluene, Benzene, Diisopropyl ether, Ethanol, Methylene chloride, Ethyl acetate, acetic acid and Formaldehyde, which were used in the Rivastigmine tartrate process. These solvents and three analytes were injected individually to confirm retention times. Rivastigmine tartrate sample solution (control sample) , Rivastigmine tartrate drug substance spiked with Methyl N-Ethyl-N-Methylcarbamate, Ethyl N-Ethyl-N-Methylcarbamate and N-Ethyl-N-Methylcarbamoyl chloride at specification level (Spiked Sample) and Rivastigmine tartrate drug substance spiked with Methyl N-Ethyl-N-Methylcarbamate, Ethyl N-Ethyl-N-Methylcarbamate and N-Ethyl-N-Methylcarbamoyl chloride and all other known residual solvents at specification level (All Spiked Sample) were injected into GCMS to confirm any co-elution of Methyl N-Ethyl-N-Methylcarbamate, Ethyl N-Ethyl-N-Methylcarbamate and N-Ethyl-N-Methylcarbamoyl chloride and Internal standard(Ethyl benzene) peaks with each other and with any other known residual solvents. The specificity results are tabulated in Table.1 and typical GCMS spectrograms of control sample, spiked sample and all spiked sample are shown in Figure.3. Based on evaluation of specificity studies, it was concluded that the Methyl N-Ethyl-N-Methylcarbamate, Ethyl N-Ethyl-N-Methylcarbamate and N-Ethyl-N-Methylcarbamoyl chloride and Internal standard (Ethyl benzene) peaks are well separated from each other as there is no other solvent co-elution indicated that the method is selective and specific for three analytes in Rivastigmine tartrate drug substance.

Table 1. Retention time details in Specificity experiment

Name	**Spiked sample**		**All spiked sample**	
	RT(min)	RRT	RT(min)	RRT
Ethyl benzene (Internal Standard)	12.495	1.00	12.497	1.00
Methyl N-Ethyl-N-Methylcarbamate	14.814	1.19	14.813	1.19
Ethyl N-Ethyl-N-Methylcarbamate	17.457	1.40	17.459	1.40
N-Ethyl-N-Methylcarbamoyl chloride	18.649	1.49	18.653	1.49

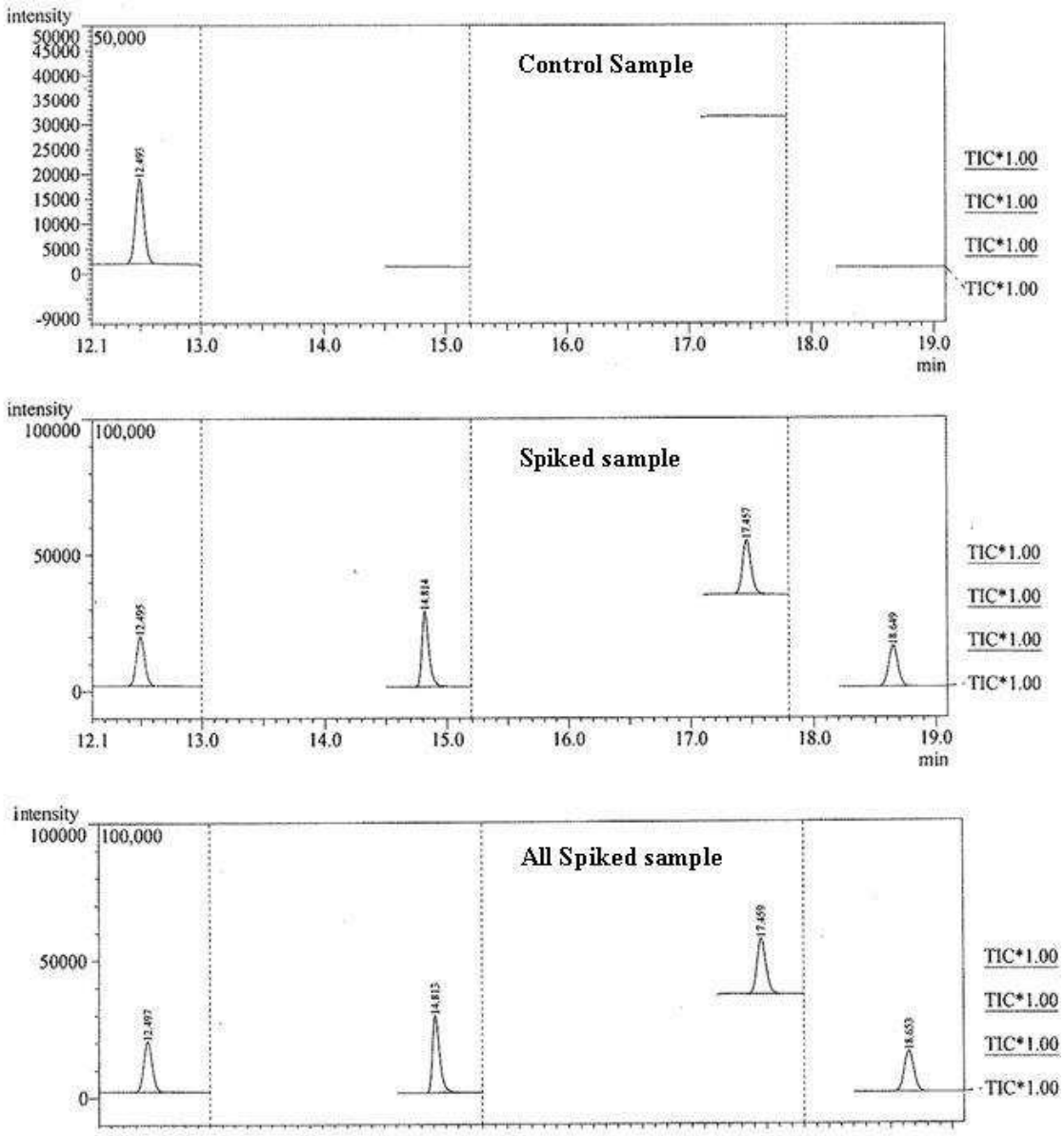


Figure 3. Typical GCMS spectrograms of control sample, spiked sample and all spiked sample

LOD & LOQ

The LOD and LOQ values of Methyl N-Ethyl-N-Methylcarbamate, Ethyl N-Ethyl-N-Methylcarbamate and N-Ethyl-N-Methylcarbamoyl chloride were predicted from Visual method. Each predicted concentration was verified for precision by preparing the solutions at about these predicted concentrations and injected each solution six times into the GCMS, results are tabulated in Table 2.

Table 2. LOD and LOQ precision results

	Area Ratio					
	Methyl N-Ethyl-N-Methylcarbamate /Ethyl benzene		Ethyl N-Ethyl-N-Methylcarbamate / Ethyl benzene		N-Ethyl-N-Methylcarbamoyl chloride / Ethyl benzene	
	LOD	LOQ	LOD	LOQ	LOD	LOQ
1	0.0249	0.0842	0.0237	0.0724	0.0226	0.0711
2	0.0245	0.0863	0.0249	0.0722	0.0228	0.0705
3	0.0241	0.0881	0.0244	0.0806	0.0230	0.0708
4	0.0248	0.0880	0.0260	0.0741	0.0231	0.0716
5	0.0252	0.0912	0.0213	0.0772	0.0238	0.0707
6	0.0230	0.0868	0.0247	0.0749	0.0229	0.0689
Mean	0.0244	0.0874	0.0242	0.0752	0.0230	0.0706
SD	0.0008	0.0023	0.0016	0.0032	0.0004	0.0009
% RSD	3.3	2.6	6.6	4.3	1.7	1.3
Con. (µg/g)	2	6	2	6	2	6

Linearity

The linearity of Methyl N-Ethyl-N-Methylcarbamate, Ethyl N-Ethyl-N-Methylcarbamate and N-Ethyl-N-Methylcarbamoyl chloride was satisfactorily done. A series of solutions were prepared by using these analytes at concentration levels from around 6 µg/g to 120µg/g(i.e LOQ to 150%). Statistical data like slope, intercept, STEYX and correlation coefficient were established by using the peak area ratio versus concentration data. Linearity results are tabulated in Table 3.

Table 3. Linearity experiment results

Methyl N-Ethyl-N-Methylcarbamate		**Ethyl N-Ethyl-N-Methylcarbamate**		**N-Ethyl-N-Methylcarbamoyl chloride**	
Conc.(µg/g)	Area Ratio (Methyl N-Ethyl-N-Methylcarbamate /Ethyl benzene)	Conc.(µg/g)	Area Ratio (Ethyl N-Ethyl-N-Methylcarbamate /Ethyl benzene)	Conc.(µg/g)	Area Ratio (N-Ethyl-N-Methylcarbamoyl chloride /Ethyl benzene)
6.0	0.0868	6.0	0.1016	6.1	0.0624
19.8	0.2656	19.7	0.2954	20.0	0.1906
39.7	0.5685	39.5	0.5927	40.1	0.4045
59.5	0.8172	59.2	0.8458	60.1	0.5713
79.3	1.1198	79.0	1.1668	80.1	0.8007
99.1	1.3617	98.7	1.4674	100.2	0.9668
119.0	1.7062	118.4	1.8282	120.2	1.2250
Slope	0.0142	Slope	0.0152	Slope	0.01
Intercept	-0.0077	Intercept	-0.0108	Intercept	-0.008
STEYX	0.023	STEYX	0.0312	STEYX	0.0221
Correlation Coefficient	0.9993	Correlation Coefficient	0.9989	Correlation Coefficient	0.9988

Precision:

The system precision of the developed method was checked by injecting standard solution for six replicates and method precision was checked by preparing the six individual sample solutions by spiking the analytes at specification level to the drug substance and injected in to GCMS. The results of system precision experiment and method precision experiment are shown in Tables 4 & 5.

Table 4. System precision experiment results

Injection ID	Area Ratio		
	Methyl N-Ethyl-N-Methylcarbamate /Ethyl benzene	Ethyl N-Ethyl-N-Methylcarbamate / Ethyl benzene	N-Ethyl-N-Methylcarbamoyl chloride / Ethyl benzene
1	1.2113	1.0559	0.9556
2	1.2132	1.1052	0.9650
3	1.2114	1.0652	0.9650
4	1.2118	1.0530	0.9602
5	1.2151	1.0971	0.9650
6	1.2174	1.1019	0.9634
Mean	1.2134	1.0797	0.9624
SD	0.0024	0.0242	0.0038
%RSD	0.2	2.2	0.4
95%Confidence Interval (±)	0.0025	0.0254	0.004

Table 5. Method precision experiment results

Sample ID	(µg/g)		
	Methyl N-Ethyl-N-Methylcarbamate	Ethyl N-Ethyl-N-Methylcarbamate	N-Ethyl-N-Methylcarbamoyl chloride
1	80	82	84
2	82	80	86
3	81	79	85
4	82	83	86
5	80	82	84
6	79	78	83
Mean	81	81	85
SD	1.2	2.0	1.2
%RSD	1.5	2.5	1.4
95% Confidence Interval (±)	1.3	2.1	1.3

Accuracy:

Standard addition experiments were conducted in triplicate preparations (i.e Rivastigmine tartrate drug substance sample solutions were prepared in triplicate by spiking with Methyl N-Ethyl-N-Methylcarbamate, Ethyl N-Ethyl-N-Methylcarbamate and N-Ethyl-N-Methylcarbamoyl chloride) to determine accuracy of the methods at LOQ to 150% of specification level and recoveries of all the analytes were determined. The accuracy experiment results are reported in Table 6.

Table 6. Accuracy experiment results

Average*	Methyl N-Ethyl-N-Methylcarbamate			Ethyl N-Ethyl-N-Methylcarbamate			N-Ethyl-N-Methylcarbamoyl chloride		
	Added (µg/g)	**Recovered (µg/g)**	**Recovery (%)**	**Added (µg/g)**	**Recovered (µg/g)**	**Recovery (%)**	**Added (µg/g)**	**Recovered (µg/g)**	**Recovery (%)**
LOQ level	5.9	6.1	103.4	5.7	5.6	98.2	5.9	6.0	101.7
50% level	39.3	40.8	103.8	37.6	39.5	105.1	39.4	42.2	107.1
100% level	78.3	81.0	103.4	74.8	80.0	107.0	78.3	85.1	108.7
150% level	117.2	114.0	97.3	112.1	114.8	102.4	117.4	120.9	103.0

**Three replicates*

CONCLUSION

The validated method is Specific, Sensitive, Linear, Precise, Accurate and Suitable for the determination of Methyl N-Ethyl-N-Methylcarbamate, Ethyl N-Ethyl-N-Methylcarbamate and N-Ethyl-N-Methylcarbamoyl chloride contents in Rivastigmine tartrate drug substance. Hence, the validated GC-MS method can be employed in to the routine analysis.

Acknowledgements
The authors gratefully acknowledge the management of APL Research Centre-II (A Division of Aurobindo Pharma Ltd.), for allowing us to carry out the present work. The authors are also thankful to the colleagues of Analytical Research Department and Chemical Research Department for their co-operation.

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