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GC-MS Method for Determination of 4-Aminophenol Impurity in Paracetamol Tablet Formulation

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Abstract - *A sensitive and selective method based on gas chromatography hyphenated to mass spectrometry (GC–MS) for the screening of developed and validated a method for the rapid analysis of impurities in paracetamol tablet formulation, by direct injection using mass detector.*

The analysis of '4-aminophenol in paracetamol tablet formation by direct injection using mass detector', have been reported for the first time. Our work deals with development of a novel gas chromatography method with mass detector which can be use to determine the content of 4-aminophenol in paracetamol tablet formulation, by direct injection using mass detector. For method validation parameters such as specificity, Non-Inference from other peaks, Limit of detection and quantification, precision, accuracy LOD and LOQ Precision etc.

*In this method validation study simple, rapid and selective chromatographic procedures were established for the simultaneous determination of 4-aminophenol in paracetamol commercially available tablets. The GC-MS method is direct, requiring minimal sample preparation and made use of the total ion current detection. The method needs no derivatization or pretreatment of the target compound. The described validated chromatographic method offer specificity advantage over the spectrophotometric based non-separation method. Finally, the proposed method was found accurate and precise, thus making them convenient for quality purposes***.**

***Key Words*: 4-Aminophenol, Gas chromatography, Mass spectrometry, Method validation, Paracetamol**

1. INTRODUCTION:

Many methods are available in literature for assay of paracetamol in diverse types of samples including pharmaceutical preparations [1-3]. These methods are as diverse as a simple titrimetric method to GC and spectrophotometric methods [4]. Owing to wide spread use of paracetamol in different kinds of pharmaceutical preparations, rapid and sensitive methods for the determination of paracetamol are being investigated. Many spectrophotometric methods of determination of paracetamol are available in literature [5-9]. These are based on hydrolysis of paracetamol to *p*-aminophenol and latter is reacted with specific reagents to produce colored substance which is monitored spectrophotometrically. Various spectrophotometric methods of analysis of paracetamol have been investigated. The method of assaying paracetamol was to react paracetamol with different reagents so that a colored species was formed, the absorbance of which was measured in visible region at appropriate wavelength [3-6]. The methods of quantitative determination of paracetamol involved the hydrolysis of paracetamol to *p*-aminophenol. Hundreds of methods are available in converting the hydrolyzed product to colored species to estimate paracetamol [6-8].

In the present work, chromatographic procedure is proposed and validated for the simultaneous determination of 4-aminophenol in paracetamol tablet formulation, by direct injection using mass detector. The method involved the application of GC–MS and the objective of the present work is to develop a simple, rapid and reliable method to assay paracetamol and to determine the 4-aminophenol in medical paracetamol tablets.

2. EXPERIMENTAL:

2.1. Instrumentation:

The GC–MS study was conducted using Shimadzu GCMS QP2020 plus gas chromatograph and with mass selective detector. The injection volume was 2 µL, and the

mass spectral scan rate was 2.50 scans per second. The GC was operated in split less mode with a carrier gas (helium grade 5), flow rate at 1.5mL/min, and a column head pressure of 10 psi. The mass spectrometer was operated on the electron impact (EI) mode using an ionization voltage of 70 eV and a source temperature of 240°C. The GC injector was maintained at 250°C and the transfer line at 280°C. The temperature program used consisted of an initial temperature hold at 60°C for 2 min, ramped up to 240°C at a rate of 10°C/min followed by a hold at 250°C for 24min. The mass spectra reported were obtained by background subtraction and are the average of at least five scans. The chromatographic separations (and collection of retention data) were carried out on a 30 m × 0.32 mm-ID column coated with 1.8 µm 100% Rtx-624.

2.2. General procedures:

GC-MS paracetamol stock solutions (1000 µg/mL) were prepared in methanol. The working solutions were prepared by dilution of the stock solutions with methanol to reach concentration ranges 4-24ppm for paracetamol. Injections were made for each concentration and chromatographed under the previously described GC conditions. The peak areas obtained from GC method was plotted against the corresponding concentrations to obtain the calibration graphs.

2.2.1. Assay of tablets:

A total of six tablets samples from six differnts manufacturers from Indian market were weighed and finely powdered. Methanol (60 mL) was added to a quantity of the powdered tablets equivalent to 100 mg paracetamol, stirred for 10 min, and then filtered into a 100mL calibrated flask. The residue was washed with 2 × 10 mL methanol, and washings were added to the filtrate and diluted to final volume with methanol. Aliquots of the tablet solution (prepared in methanol) were diluted with methanol to obtain final concentrations within the previously mentioned ranges and then treated as under the procedures for GC–MS method [10-11].

2.2.2. Specificity:

The specificity of the method was assessed by analyzing blank, 4-aminophenol standard, and paracetamol standard and tablet sample of paracetamol. This was done to investigate whether there had been any interference from the endogenous substances and evaluate the interference of commonly used medicine for paracetamol certain medications were added to the blank methanol samples, which were analyzed [12-13] by GC-MS.

2.2.3. Linearity and range:

Linearity was studied by diluting stock standard solutions of paracetamol with methanol solvent to give a concentration range of 4 to 24 ppm. Calibration curve of absorbance vs concentration was plotted using standard solutions of 4ppm to 24ppm and regression line equation and correlation coefficient was determined [14]. The range of solution has been decided according to statistical analysis of regression equation [15].

2.2.4. Stock Solution Stability:

The stability of the stock solutions of paracetamol was evaluated at room temperature up to 24 hours. The freshly prepared solutions were injected and then kept at room temperature. These samples were analyzed after 12 and 24hours respectively. At each time interval i.e. at 0, 12 and 24 hours the solutions were injected in triplicate.

2.2.5. Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions [16]. Precision of the method was studied by intra- and inter-day variations method. Intraday precision was evaluated by assaying six different sample preparations on the same day. Interday precision was performed by assaying six different sample preparations on different days at different time intervals. The percentage relative standard deviation (% RSD) was calculated (**Table-1** and **3**).

2.2.6. Accuracy*:*

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found and this is sometimes termed trueness [16]. The method was applied to drug sample and accuracy of the method was determined by calculating recovery of paracetamol at 80%, 100% and 120% level of label claim. Percentage recovery was calculated using equation for the method and the results are presented in (**Table-4**).

3. RESULTS AND DISCUSSION:

Mass spectrum of paracetamol (**Fig-1)** is characterized by base peak at *m/z* 109 formed by hydrogen transfer from methyl group of the acetyl moiety to the ionized nitrogen followed by α-cleavage. Subsequent rearrangement followed by the

loss of the formaldehyde radical results in the formation of the more stable conjugated cyclopentadienylidene ammonium cation at *m/z* 80. Ionization of the amide oxygen followed [17] by α-cleavage gave the CH_3CO cation at *m/z* 43. Structure of the paracetamol fragmentation ions are shown in the **Fig-1**. Further proof of the suggested pathways was possible after the preparation of paracetamol from condensation of 4-aminophenol and acetic anhydride. The mass spectrum in a **Fig-1** shows the analogous peaks at 110, 81 and 46 respectively.

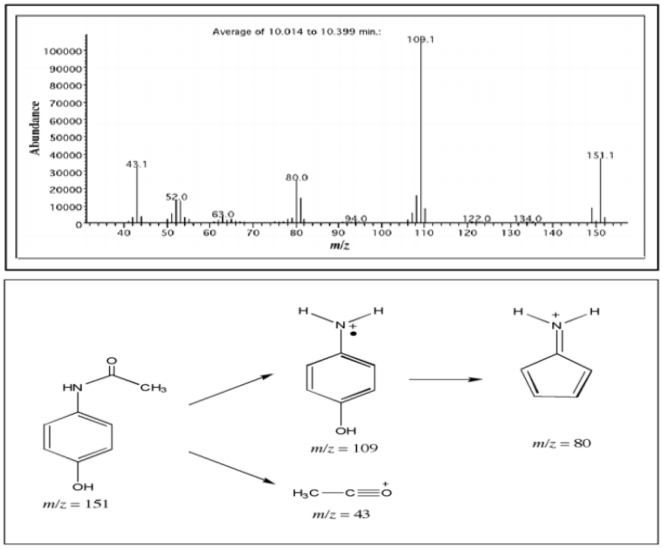
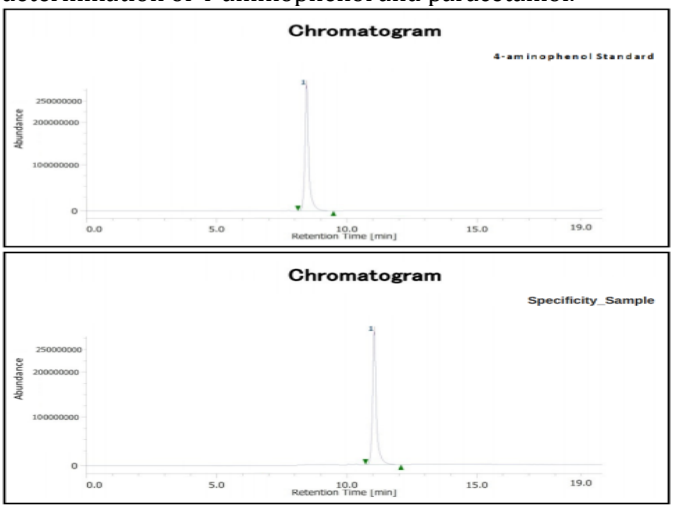


Fig-1: Mass spectrum of paracetamol standard and fragmentation pattern of paracetamol

3.1. Specificity:

Fig-1 and **Fig-2**, the retention time of 4-aminophenol and paracetamol are 8.27 and 10.90 minutes respectively, it was found that there is no interference at the retention time of 4-aminophenol and paracetamol from any of the blank and hence the proposed method is specific for the determination of 4-aminophenol and paracetamol.



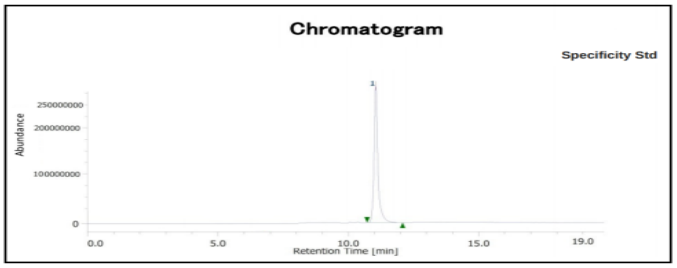


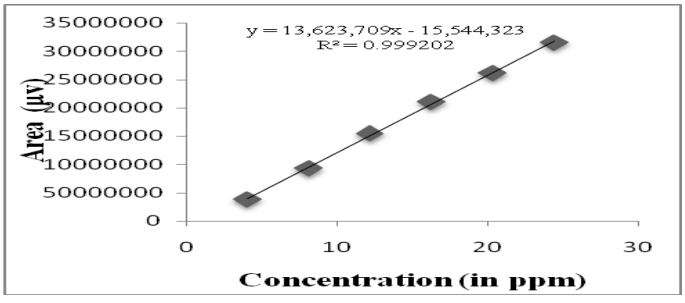
Fig-2: GC-MS chromatograms of 4-aminophenol standard, paracetamol standard and sample

3.2. Linearity (Working range):

This experiment was conducted to demonstrate the working range over which the response of the detector is linear with respect to the concentration of paracetamol. A series of standard solutions of varying concentrations ranging from 4.07–24.42 ppm were prepared.

Table-1: Preparation of paracetamol and linearity data standard for linearity

Linearity Solution Level	Vol. of Stock solution mL)	Diluted to (mL)	Conc ppm	Replications	Peak Area Counts	Means Area
L1	0.4	20	4.07	R1	38443124	38449952
				R2	38456781	
L2	0.8	20	8.14	R1	92886248	92737731
				R2	92589214	
L3	1.2	20	12.21	R1	154594963	154610376
				R2	154625789	
L4	1.6	20	16.28	R1	209772496	209813570
				R2	209854644	
L5	2	20	20.35	R1	260746802	260873584
				R2	261000365	
L6	2.4	20	24.42	R1	318438939	314667280
				R2	310895621	



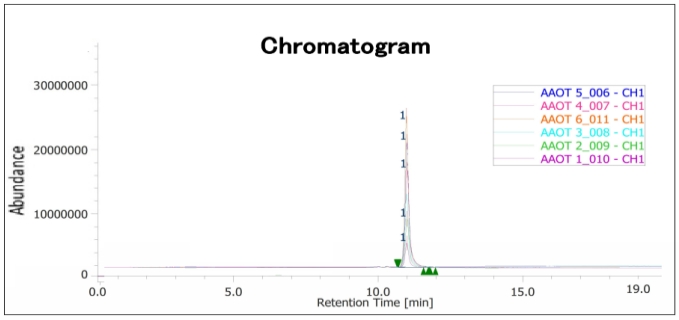


Fig-3: Linearity Overlay and graphs of paracetamol standard

Slope	13623709.294489
Intercept	-15544323.4
correlation [r]	0.999600723
R^2	0.999202

Regression Analysis:

The calibration data were subjected to regression analysis [18-19].

The regression equation is Y = mX + C and can be written as,

$$Y = 13623709x - 15544323$$

Acceptability of linearity data was judged by examining the coefficient of determination, the y-intercept and the response factors as follows,

a) A plot of concentration Vs peak area response shows that the response is linear in the concentration range of 4.07 to 24.42ppm.

b) The coefficient of regression i.e. r^2 is 0.999, means that 99.9% of the variation in Y i.e., the change in the response of the analyte can be explained by changes in the X i.e., the concentration of the analyte. The correlation coefficient is a measure of the goodness of the fit of the calculated line to the sample data.

c) The y-intercept for this particular range is 0.000 as its passing through origin. It is concluded from the plot of response versus concentration of indicates a linear response is obtained in this concentration range.

3.3. Stock Solution Stability:

From the results tabulated in **Table-2**, it can be seen that the percentage difference is not more than 2.0% indicating that the results obtained after the specified storage temperatures are comparable with the initial results. It can be concluded that the solutions are stable for 24 h at room temperature.

Table-2: Standard solution stability at 0, 12 and 24 hrs

Inj. No	Interval	Area (µv)	Mean Area (µv)	Mean % Content	Rel Diff (%)
1	0 hr	260742456	260998796.7	99.74	NA
2		261002456			
3		261251478			
1	12 hrs	260507933	260430944.7	99.52	0.22
2		260769952			
3		260014949			
1	24 hrs	260014748	260013364.7	99.36	0.38
2		260014235			
3		260011111			

3.4. Precision:

3.4.1. Injection Repeatability:

The percentage relative standard deviation for all the above parameters is below 2.0. The low values of standard deviation and coefficient of variation indicate good repeatability. It also indicates the satisfactory performance of the system and system suitability for the method [20].

Table-3: Precision injection repeatability for paracetamol tablet formulations.

Sample no.	Conc in ppm	Area (mv)	% Content
Sample-1	20.32	263751151	100.82
Sample-2	20.36	262762541	100.24
Sample-3	20.42	261749875	99.56
Sample-4	20.4	261745690	99.66
Sample-5	20.3	260751151	99.77
Sample-6	20.4	261751169	99.66
Average	NA	NA	99.95
STDEV	NA	NA	0.49
% RSD	NA	NA	0.49

3.4.2. Intra-Assay Precision:

The relative standard deviation of the intermediate precision results should not be more than 2.0%. The absolute difference between the mean value of method precision and intermediate precision should not be more than 2.0%.

Table-4: Intra-assay precision of paracetamol for paracetamol tablet formulations

Sample no.	Conc in ppm	Area (mv)	% Content
Sample-1	20.02	246761965	98.63
Sample-2	20.10	247752003	98.63
Sample-3	20.20	248741365	98.53

Sample-4	20.23	249765690	98.79
Sample-5	20.11	249300900	99.19
Sample-6	20.05	249262459	99.48
Average	NA	NA	98.88
STDEV	NA	NA	0.38
% RSD	NA	NA	0.38

Table-5: Comparison between analyst-1 and 2

	Mean % Content	Absolute Difference
Analyst 1	99.95	1.08
Analyst 2	98.88	

The mean, standard deviation and percentage relative standard deviation was calculated [21-23]. The peak area was comparable to those of the intra-assay results. The results are in **Table-3,** and **4**. From the precision results, it can be concluded that the method is repeatable, while the intermediate precision results show that the method is reproducible by different analysts on different day also. The relative standard deviation of the intermediate precision results should not be more than 2.0%. The absolute difference between the mean value of method precision and intermediate precision should not be more than 2.0%.

3.5. Accuracy (Recovery Experiment):

The accuracy experiment was conducted by the standard addition method. This experiment was conducted to determine the accuracy of the method in terms of percentage recovery. The experiment was performed by fortifying three known concentrations of sample into three solutions at different levels and assayed for active ingredient content. Known amounts of pure standard were added to the paracetamol tablet formulation sample at three levels and accuracy in terms of percentage recovery was determined from the amount found versus added or fortified [24].

The percentage recovery for the fortification of paracetamol tablet formulation should be in the range of 98-102%. Result of accuracy (% recovery) of paracetamol tablet formulation is tabulated in Table VI. The percent recovery of the added standard was found to be between 99.62 –100.19 % and with the overall % mean recovery of 99.88. This indicates that the method is free from either positive or negative interferences from the blank. From the above result, it was found that the recovery data of the analyte is within the limit and hence the proposed method is accurate[25].

Table-6 Accuracy data of paracetamol

Level (%) / pptn	Smpl Wt (in mg)	Conc (in ppm)	Area (µv)	% Recovery	% Mean Recovery
80_1	16.02	10.02	204915789	100.25	99.83
80_2	16.15	10.15	204795461	99.38	
80_3	16.06	10.06	204615789	99.85	
100_1	20.05	20.05	256435690	100.23	100.19
100_2	20.14	20.14	256356978	99.76	
100_3	19.98	19.98	256445680	100.59	
120_1	24.31	30.31	310789561	100.19	99.62
120_2	24.47	30.47	310756980	99.53	
120_3	24.56	30.56	310659874	99.13	
Overall % Recovery				99.88	
Overall STDEV				0.48	
Overall % RSD				0.48	

3.6. Range:

The range of an analytical procedure is the interval between the lower and upper concentration (amounts) of the analyte in the sample, for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity [26-27]. The range for the paracetamol is evaluated from 20% i.e. 4 ppm to 150% i.e. 24 ppm.

Solution	20% (4 ppm)	120% (24 ppm)
1	38443124	312438939
2	38456781	310895621
3	38470438	310659874
4	38484095	310552413
5	38497752	310662880
6	38511409	310773348
Average	38477266.5	310997179.2
STDEV	25549.90747	715881.7669
% RSD	0.07	0.23

Table-7: Range for paracetamol tablet formulations

3.7. Force degradation Studies:

Table-8: Forced degradation calculation of paracetamol

% Content						
Condition	Smpl Wt (in mg)	Conc. (in ppm)	Area (µv)	% Assay	% Total Imp.	Mass Balance
As such	20.07	20.07	255462581	99.75	0.000	NA
0.05M_FeCl3_2	20.03	20.03	255567894	99.99	0.138	100.4

4 Hrs						
1N_NaOH_24 Hrs	20.17	20.17	256478912	99.65	0.618	100.5
1N_HCl_24 Hrs	20.09	20.09	254256781	99.18	0.112	99.5
3% H2O2_24 Hrs	20.11	20.11	257457894	100.33	0.826	101.4
1% Na2S_24 Hrs	19.95	19.95	256114523	100.61	0.826	101.7
Photo @ 1.2 million lux/Hr	19.96	19.96	254462581	99.91	0.657	100.8
Thermal @ 105°C_24 Hrs	20.03	20.03	253456892	99.17	0.000	99.4

Table-9: Force degradation of paracetamol of impurity profile

RT about -->	Unk @ 2.90	Unk @ 3.15	Unk @ 3.26	Unk @ 3.45	Unk @ 4.82	Unk @ 4.82	Unk @ 6.32	Total Imp
As such	ND	ND	ND	ND	ND	ND	ND	0.000
0.05M_$FeCl_3$_24 Hrs	ND	ND	ND	ND	ND	0.115	ND	0.139
1N_NaOH_24 Hrs	0.09	ND	0.235	0.254	ND	ND	ND	0.618
1N_HCl_24 Hrs	ND	0.023	ND	ND	0.059	ND	ND	0.112
3% H_2O_2_24 Hrs	ND	ND	0.826	ND	ND	ND	ND	0.826
1% Na_2S_24 Hrs	ND	ND	0.826	ND	ND	ND	ND	0.826
Photo @ 1.2 million lux/Hr	ND	ND	ND	ND	ND	ND	0.657	0.657
Thermal @ 105°C_24 Hrs	ND	ND	ND	ND	ND	ND	ND	0.000

3.7. Analysis of paracetamol tablet formulation:

Assay of paracetamol tablets formulations: A five tablets samples from five different manucatures have been bought from the Indian market such as Wellona pharma, Schwitz Biotech, Bhumi Pharmaceuticals, Lifevision Healthcare and Medlab Pharmaceuticals Private Limited etc. were weighed and finely powdered. Methanol was added to a quantity of the powdered tablets equivalent to 20 mg, stirred for 10 min, then filtered. Aliquots of the tablets solutions (prepared in methanol) were diluted with methanol (for GC measurement) to obtain final concentrations within the previously mentioned ranges and then treated as under the procedures for GC–MS method [28].

The developed chromatographic methods were applied for the assay of the paracetamol in their combined pharmaceutical formulation. **Table-10** shows the results obtained for the proposed methods as well as the reference derivative spectrophotometric method. The assay results showed good precision and accuracy as indicated from % recovery, SD, and RSD (%) values. No interfering peaks were observed in the GC chromatograms of the tablets. Results obtained by the developed methods were statistically compared with those of the previously published derivative spectrophotometric method using single factor analysis of variance (ANOVA) test, which is considered a useful statistical tool for comparison of recovery data obtained from proposed method[29]. The calculated assay values, indicating that, no 4-aminophenol impurity is detect in the five different samples from market tablet formulations.

Table-10: Analysis of paracetamol tablet formulation

Sample No	Smpl Wt (in mg)	Conc (in ppm)	Area (mv)	% Assay	% Metformin	Mass Balance
Wellona Pharma	20.12	20.12	255446581	99.50	0.000	99.5
Schwitz Biotech	20.14	20.14	256457891	99.80	0.000	99.80
Bhumi Pharmaceuticals	20.19	20.19	257465899	99.94	0.000	99.94
Lifevision Healthcare	20.09	20.09	254266478	99.19	0.000	99.19
Medlab Pharmaceuticals Private Limited	20.11	20.11	256458794	99.94	0.000	99.94

4. CONCLUSION:

In this method validation study simple, rapid and selective chromatographic procedures were established for the simultaneous determination of 4-aminophenol in paracetamol commercially available tablets. The GC-MS method is direct, requiring minimal sample preparation and made use of the total ion current detection. The method needs no derivatization or pretreatment of the target compound. The described validated chromatographic method offer specificity advantage over the spectrophotometric based non-separation method. Finally, the proposed method was found accurate and precise, thus making them convenient for quality purposes.

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