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Simultaneous Determination of Cefotaxime Sodium and Paracetamol by LC-MS

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ABSTACT: *A rapid and sensitive liquid chromatography-mass spectrometry (LC-MS) method has been developed and validated for simultaneous determination of cefotaxime sodium and paracetamol. Sulfamethoxazole was used as the internal standard. A phenomenex Luna C_{18} column provided chromatographic separation of the analyte which was followed by detection with mass spectrometry. The mass transition ion-pair was followed as m/z 152.1 for paracetamol and 478.1 m/z for cefotaxime sodium. The mass transition of internal standard sulfamethoxazole was found at 254.0 m/z. The method involves simple isocratic chromatography conditions with mobile phase acetonitrile:20mM ammonium acetate buffer in the ratio of 40:60 and mass spectrometric detection that enables detection at ppm levels. The retention times were 3.477 and 5.601 min for Cefotaxime sodium and paracetamol respectively. The proposed method has been validated with linear range of 1-40 ppm for both the drugs. The precision values are ≤ 2 %. The accuracy of the method was found to be 98-102 %.*

KEY WORDS: *Cefotaxime sodium, Paracetamol, Sulfamethoxazole, LC-MS.*

I. INTRODUCTION

Cefotaxime Sodium, chemically is sodium 7-[2-(2-amino-4-thiazolyl) glyoxylamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo oct-2-ene-2-carboxylate 72(Z)-(omethyloxime), acetate (ester) with molecular formula[1] $C_{16}H_{17}N_5O_7S_2$. Cefotaxime is a third-generation parenteral. Cephalosporin antibacterial used in the treatment of infections due to susceptible Gram-positive and Gram-negative bacteria, including infections of the abdomen, bones and joints, CNS, skin and skin structure, genito-urinary tract (including gonorrhoea) and respiratory tract, in gynaecological infections, and in early Lyme disease[2,3]. Literature survey revealed that several HPLC [4-11], HPTLC [12] and spectrophotometric methods [13-18] have been used for determination of Cefotaxime Sodium. Paracetamol is chemically 4-hydroxy acetanilide. It is a weak inhibitor of peripheral cyclooxygenase and its analgesic effects may arise from inhibition of prostanoid synthesis in the CNS. The antipyretic effects of paracetamol are due to its action at the level of the hypothalamus to reduce pyrogen-initiated alterations in body temperature by inhibiting prostaglandin synthesis [19, 20]. Analysis of Paracetamol tablet was reported by UV spectroscopy, HPLC and HPTLC. The UV spectroscopy and RP-HPLC method were also developed for the analysis of Paracetamol in combined dosage form [21-23]. Usage of these two drugs simultaneously may result in drug-drug interactions. The analytical method reported requires laborious extraction procedure like liquid liquid extraction or solid-phase extraction (SPE) involving drying and reconstitution, long run time and high quantification limit. It is necessary, therefore, to develop a simple, specific, rapid and sensitive analytical method for the quantification of the Cefotaxime sodium and paracetamol. This paper describes development and validation of a simple, specific, rapid and sensitive LC-MS method for the simultaneous determination of cefotaxime sodium and paracetamol. So, this developed method is useful for the study of drug-drug interactions in human plasma with optimised conditions.

II. EXPERIMENTAL

The reference standards of cefotaxime sodium, paracetamol, sulfamethoxazole were obtained from, Micro Labs (Bangalore, India). High purity water was prepared in-house using a Milli-Q water purification system obtained from Millipore (India) Pvt. Ltd. (Bangalore, India). HPLC grade acetonitrile was purchased from E. Merck Ltd. (Mumbai, India).

2.1 Calibration curves: The stock solutions of cefotaxime sodium, paracetamol and sulfamethoxazole were prepared in methanol at free base concentration of 1000 µg mL. Different mobile phases of methanol:water (30:70 %, v/v), acetonitrile:water (70:30, 80:20 %, v/v), acetonitrile:20mM ammonium acetate buffer (50:50) were used in different compositions of mobile phases at different flow rates (1.0,1.2, 1.5, 1.8 mL/min). The composition of the mobile phase acetonitrile:20mM ammonium acetate buffer in the ratio of 40:60 % v/v at flow rate of 1.0 mL/min gave sharp peaks with minimum tailing and good resolution for cefotaxime sodium, paracetamol and internal standard whereas broad peaks and pronounced tailing

was observed with other compositions of mobile phases and other flow rates. Blank methanol was screened prior to experiment to ensure it was free of endogenous interference at retention times of the drugs selected. The calibration curve ranged from 1 to 40 ppm.

III. INSTRUMENTATION

Chromatographic separation was carried out in Shimadzu HPLC with phenomenex Luna C_{18} (250 × 4.6 mm id, 5µ) column. Quantitative LC-MS was performed on a Agilent LC-MSD Trap SL mass spectrometer (Waldron, Germany) equipped with electrospray ion interface, operating in positive ion polarity with peak scientific-nitrogen generator, Galilio-Roughing vacuum pump. Nitrogen gas was used as nebulizer and curtain gas. Collision induced dissociation was achieved using helium gas. MDS Sciex-electron for source ionization. The output signal was monitored and integrated using Analust 1.4 vesion software.
A mobile phase consisting of a mixture of acetonitrile and 20mM ammonium acetate buffer in 40:60% v/v ratio, pumped at a flow rate of 1.0 mL/min and detection wavelength was 254 nm. The injection volume was 20 µL and the total analysis time per each combination of sample(cefotaxime sodium, paracetamol and internal standard) was 20.0 min. The spray voltage and capillary temperature were 1.3 KV and 400 ºC, respectively. The data acquisition was ascertained by LC-MS solution data station.

IV. VALIDATION

The method was validated for selectivity, sensitivity, linearity, precision, accuracy and stability. The selectivity of the method was evaluated by comparing the chromatograms obtained from the samples containing cefotaxime sodium, paracetamol and the internal standard with those obtained from blank samples. Sensitivity was determined in terms of LLOQ (lower limit of quantification) where the response of LLOQ was at least five times greater than the response of interference in blank at the retention time or mass transitions of the analyte. For linearity, different concentrations of standard solutions were prepared to contain 1 ppm to 40 ppm of all three drugs. These solutions were analyzed and the peak areas and response factors were calculated. The calibration curve was plotted using response factor vs. concentration of the standard solutions.

Standard curve fitting was determined by applying the simplest model that adequately describes the concentration-response relationship using appropriate weighing and statistical tests for goodness of fit. The precision of the method was determined by intraday precision and interday precision. The intra-assay precision and accuracy was calculated for 6 replicates at each lower limit of quantification (LLOQ), low quality control (LQC), middle quality control (MQC) and high quality control (HQC) levels, each on the same analytical run and inter-assay precision and accuracy was calculated after repeated analysis in three different analytical runs. Accuracy of the developed method was determined by relative and absolute recovery experiments. The relative recovery of the drug was calculated by comparing the amount of the drug obtained from the drug supplemented with the actually added amount. Recovery studies were carried out for three levels at 6 times and the percentage recovery, mean, standard deviation and coefficients of variation were calculated.

V. RESULTS AND DISCUSSION

5.1 Method development: The objective of this work is to develop and validate a simple, rapid and sensitive assay method for the simultaneous quantification of cefotaxime sodium and paracetamol. To achieve the objective, different options was evaluated to optimize detection parameters and chromatography during method development. The standard solutions of cefotaxime sodium and paracetamol were analyzed by LC-MS system using direct injection probe with ESI and APCI interfaces. From the mass spectrum recorded, the detection molecular ion selected was for paracetamol as m/z 152.1 for and 478.1 m/z for cefotaxime sodium. The mass transition of internal standard sulfamethoxazole was found at 254.0 m/z.

5.2 Optimization of the chromatographic conditions: Optimization of the chromatographic conditions are intended to take into account the various goals of method development and to weigh each goal (resolution, run time, sensitivity, peak symmetry, etc.) accurately, according to the requirement of LC-MS and HPLC methods being used for the simultaneous estimation of drugs. Different mobile phases, namely, acetonitrile, methanol and ammonium acetate buffer in aqueous phase were used at a flow rate of 0.5, 0.8 and 1.0 mL/ min. For the initial separation conditions, methanol was used because of its favourable UV transmittance, low viscosity and better solubility. When methanol was substituted by other solvents, the solvents to buffer ratios were calculated using solvent strength. The resulting ratios of the mobile phase were prepared and the drugs chromatographed. These mobile phases gave well retained and symmetrical peaks. The standard solution was chromatographed with mobile phases of different ratios of organic and aqueous phases at a flow rate of 1 mL/min and 0.8 mL/min. Acetonitrile:20mM ammonium acetate buffer in the ratio of 40:60 was selected as the

mobile phase. Reversed phase C_{18} stationary phases were used and the chromatograms were recorded. Based on the retention and peak shape, Phenomenex Luma C_{18} column was selected for analysis. Typical mass spectrum and chromatogram of cefotaxime sodium, paracetamol and sulfamethoxazole are given in

Fig. 1 and 2 respectively.

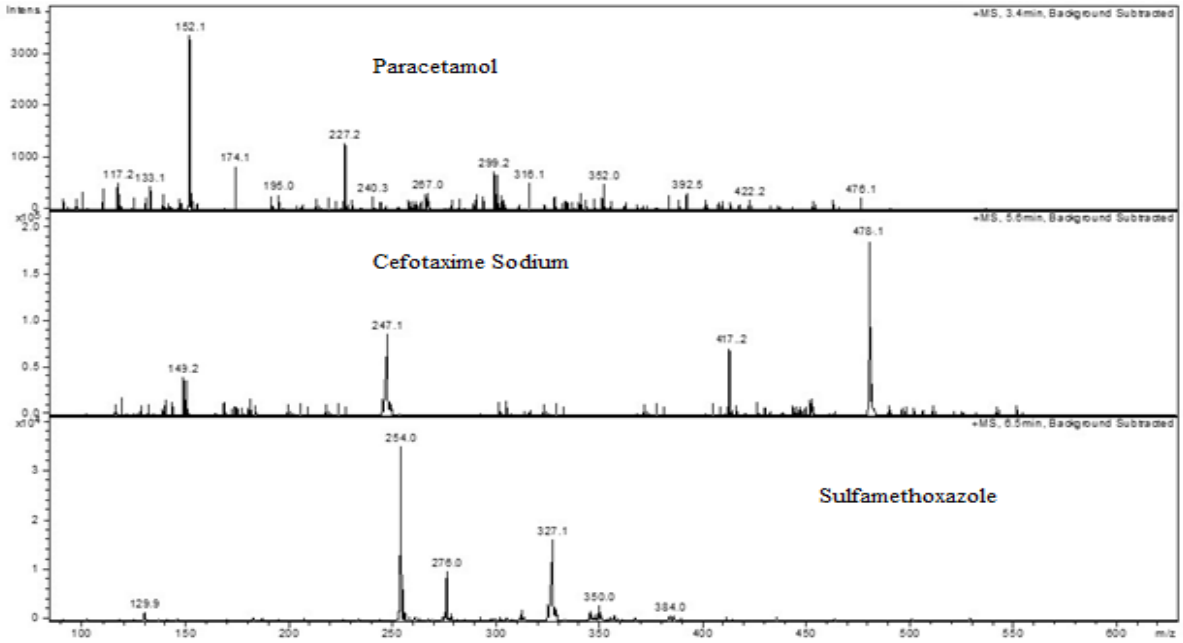

Fig.1 Mass spectrum of Paracetamol, Cefotaxime sodium and Sulfamethoxazole (IS)

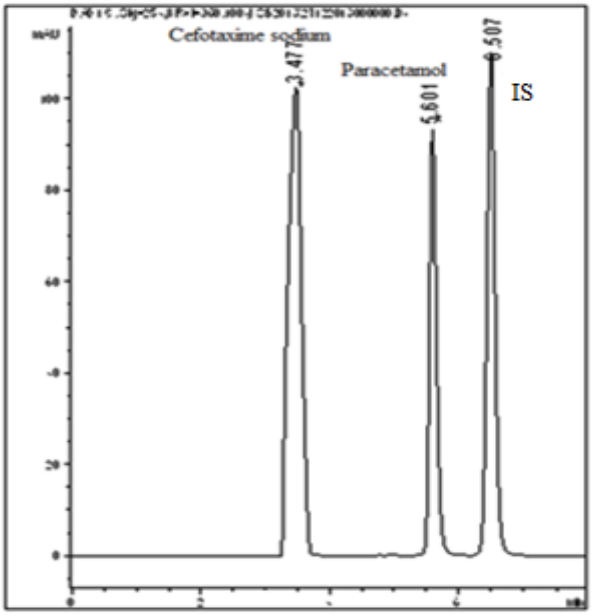

Fig.2 LC-MS chromatogram of Cefotaxime sodium, Paracetamol and Sulfamethoxazole (IS)

5.3 Validation: simultaneous estimation of cefotaxime sodium, paracetamol was carried out using optimized chromatographic conditions. The validation parameters such as accuracy, precision (repeatability and reproducibility), linearity and range, sensitivity (limit of detection and limit of quantitation) robustness/ruggedness, selectivity/specificity and system suitability were evaluated. The optimized conditions are given in TABLE 1.

TABLE 1: Optimized Chromatographic Conditions

Parameters	**Method**
Stationary phase (column)	Phenomenex Luma C_{18} (250 x 4.6 mm, 5 µm packed with 5 µ)
Mobile Phase	Acetonitrile:20mM ammonmmium acetate buffer (40:60)
Column temperature (^{0}C)	Ambient
Volume of injection loop (µL)	20
Drug-1 (cefotaxime sodium)	478.1 m/z
Drug-2 (paraceatmol)	152.1 m/z
Internal standard (Sulfemethoxazole)	254.0 m/z
Polarity	Positive
Internal standard (IS)	Sulfamethoxazole
Drug-1 RT (min)	3.477
Drug-2 RT (min)	5.601
ISTD RT (min)	6.507
Run time	8 min

5.3.1 Linearity: It was observed that the optimized methods were linear within a specific concentration range for cefotaxime sodium and paracetamol. The calibration curves were plotted between response factor and concentration of the standard solutions. The linearity ranges were found to be 1 to 40 ppm. The results indicated no significant interday variability of slopes and intercepts over the optimized concentration range. The linearity chromatograms were shown in the Fig. 3 and 4.

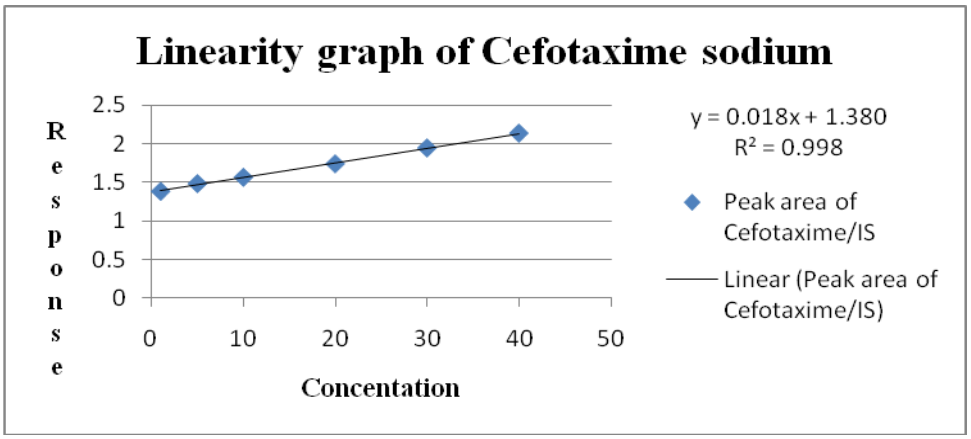

Fig.3 Linearity graph of Cefotaxime sodium

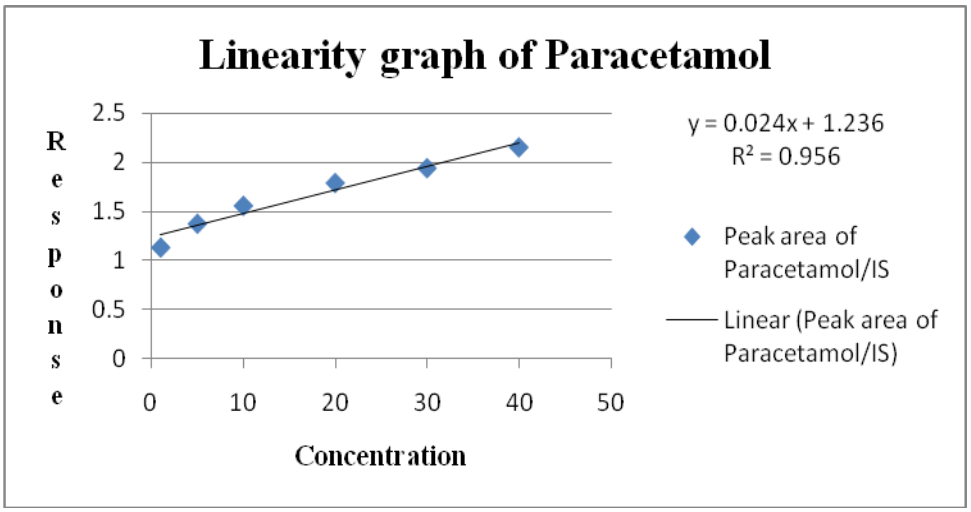

Fig.4 Linearity graph of Cefotaxime sodium

5.3.2 Accuracy: The accuracy of the optimized methods was determined by relative and absolute recovery experiments. The percentage recovery values for the drugs ranged from 98-102%. The coefficient of variation (%) of these values was less than 10.00 %. It is indicative that the developed methods are accurate and reliable. Recovery data of the selected drugs is given in TABLE 2.

TABLE 2: Recovery of cefotaxime sodium, paracetamol and sulfamethoxazole

	LQC (1 ppm)			**MQC (20 ppm)**			**HQC (40 ppm)**		
	Drug 1	**Drug 2**	**IS**	**Drug 1**	**Drug 2**	**IS**	**Drug 1**	**Drug 2**	**IS**
% Recovery	100.07	99.81	100.12	98.83	99.40	100.03	98.46	100.12	100.26
% RSD	0.334	0.348	0.315	0.441	0.337	0.339	0.349	0.464	0.321
% CV	0.061	0.12	0.057	1.01	0.45	0.023	0.99	0.094	0.11

Drug 1- cefotaxime sodium, Drug 2-paracetamol IS- sulfamethoxazole

From the linearity it was found that the drug obeys linearity within the concentration range of 1-40 ppm. From the results shown in recovery (Table-2), it was found that the recovery for drug and internal standard was reproducible.

5.3.3 Precision: The optimized method for the simultaneous estimation of cefotaxime sodium and paracetamol was found to be precise. This was evident from the coefficient of variation values, which were less than 10.00 % at all concentrations and the %RSD were found to be ≤ 2%. Precession study of cefotaxime sodium is given in

TABLES 3 and 4. Precession study of paracetamol is given in TABLES 5 and 6.

TABLE 3: Precession study of Cefotaxime sodium (Intraday)

Precision	**QC Sample (ng/mL) (n= 6)**	**Concentration found**	**SD**	**CV (%)**
LQC	1.00	0.998	0.006	0.623
MQC	20.00	19.98	0.0091	0.916
HQC	40.00	39.89	0.012	1.102

TABLE 4: Precession study of Cefotaxime sodium (Interday)

Precision	**QC Sample (ng/mL) (n= 6)**	**Concentration found**	**SD**	**CV (%)**
LQC	1.00	0.986	0.014	0.129
MQC	20.00	19.81	0.021	0.214
HQC	40.00	39.63	0.165	1.511

TABLE 5: Precession study of Paracetamol (Intraday)

Precision	**QC Sample (ng/mL) (n= 6)**	**Concentration found**	**SD**	**CV (%)**
LQC	1.00	0.996	0.003	0.291
MQC	20.00	19.95	0.0051	0.503
HQC	40.00	39.93	0.009	0.917

TABLE 6: Precession study of Paracetamol (Interday)

Precision	**QC Sample (ng/mL) (n= 6)**	**Concentration found**	**SD**	**CV (%)**
LQC	1.00	0.991	0.005	0.542
MQC	20.00	19.84	0.0079	0.760
HQC	40.00	39.86	0.012	1.206

5.3.4 Selectivity: The selectivity of the method was evaluated by comparing the chromatograms obtained from the samples containing cefotaxime sodium and paracetamol and the internal standard with those obtained from blank samples. These chromatograms were compared with the chromatograms obtained from standard solutions. Each chromatogram was tested for interference. The combination of the sample preparation procedure and chromatography provided an assay which is free from significant interfering endogenous components at the retention times of the selected drugs and the internal standard. These observations show that the developed assay method is specific and selective. System suitability date of selected drugs is given in TABLES 7 and 8.

TABLE 7: System suitability of Cefotaxime sodium

Replicate Injection No	Retention time		Area Ratio
	Analyte	Internal Standard	
1	3.477	6.507	1.291
2	3.477	6.507	1.289
3	3.477	6.507	1.293
4	3.477	6.507	1.289
5	3.477	6.506	1.291
6	3.477	6.507	1.292
MEAN	3.477	6.5068	1.2908
SD	0.000	0.00048	0.0016
%CV	0.00	0.000	0.0012

TABLE 8: System suitability of Paracetamol

Replicate Injection No	Retention time		Area Ratio
	Analyte	Internal Standard	
1	5.601	6.507	1.092
2	5.601	6.507	1.091
3	5.601	6.507	1.089
4	5.601	6.507	1.092
5	5.601	6.506	1.087
6	5.601	6.507	1.093
MEAN	5.601	6.5068	1.0907
SD	0.000	0.00048	0.0023
%CV	0.00	0.000	0.002

5.3.5 Limit of detection and Limit of quantification: The limit of detection (LOD) value was found to be 0.301 ppm for cefotaxime sodium and 0.294 ppm for paracetamol and their limit of quantification (LOQ) values were 0.993 for cefotaxime sodium and 0.97 ppm.These observations indicate that the developed methods have adequate sensitivity. This value, however, may be affected by the separation conditions (e.g., column, reagents and instrumentation and data systems), instrumental changes (e.g., pumping systems and detectors) and use of non HPLC grade solvents and may result in changes in signal to noise ratios.5.3.6 Ruggedness: The ruggedness of the method was studied by changing the experimental conditions. No significant changes in the chromatographic parameters were observed when changing the experimental conditions (operators, source of reagents and column of similar type) and optimized conditions (pH, mobile phase ratio and flow rate).

VI. CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for simultaneous determination of cefotaxime sodium and paracetamol. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of other endogenous materials in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of cefotaxime sodium and paracetamol using LC-MS.

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