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Concurrent Assay of Acetaminophen, Pseudoephedrine, Guaifenesin and Dextromethorphan in Formulations by Packed Column Supercritical Fluid Chromatography

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The isocratic separation and simultaneous estimation of acetaminophen, pseudoephedrine hydrochloride, guaifenesin and dextromethorphan hydrobromide in bulk and pharmaceutical dosage forms using the technique of packed column supercritical fluid chromatography (pcSFC), has been described. Method development was based on the measurement of retention times as a function of parameters of pressure, temperature and modifier composition. An arbitrary mixture of the 4 drugs was base-line resolved on a Hypersil-Phenyl (250 x 4.6 mm) 5 µm column with a ternary mobile phase of carbon dioxide modified with 14.29% (v/v) methanol containing 1% methylamine. Detection was absorptimetric at 210 nm. The method has been fully validated and a full statistical evaluation included. Application of the method to real-time samples revealed no interferences from excipients. A comparison of the method with an HPLC method for the same assay, which appeared in literature, validated the recent proposition that pcSFC is not only a viable but also a superior alternative to HPLC for pharmaceutical analysis.

Pharmacopoeia of developed countries such as USA, UK and EU have recognised RP-HPLC/UV as a main method of analysis. At the same time they maintain that there is an urgent need for a cleaner method of assay involving non-toxicity and reduced environmental hazards. Packed column supercritical fluid chromatography (pcSFC) using modified supercritical fluid carbon dioxide as the mobile phase is one such clean method, the capabilities of which are worth exploring. Recent literature on this subject gives credence to the above statement[1-7]. The present paper describes a pcSFC method for the isocratic separation and simultaneous assay of a multi-drug composition, commonly used for the management of common cold.

The formulation contains acetaminophen, guaifenesin, pseudoephedrine and dextromethorphan. Literature cites several conventional and instrumental (spectrophotometry, HPLC) methods for the assay of the 4 individual drugs. However no method has been reported for the separation and assay of these drugs in combination, except for a recently reported HPLC method[8]. The method is cumbersome and tedious compared to the present method. In order to establish the viability and advantages of pcSFC over HPLC, a comparison of this method with the present method is provided.

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MATERIALS AND METHODS

Stock solutions (1 mg/ml) of the individual drugs were prepared by dissolving 100 mg of each drug in 100 ml of methanol. Appropriate concentrations of the drugs and mixtures were obtained by further dilutions. The working standards of the drugs were obtained from the Pharma companies with certificates of analysis. Methanol, HPLC Grade was obtained from M/s. E. Merck (India) Ltd.

Carbon dioxide (99.9% pure) was obtained from M/S Bombay Carbon Dioxide Ltd. Mumbai.

SFC apparatus:

The apparatus used was a JASCO supercritical fluid chromatograph of 900-series configured, for dynamic mixing with a 2-pump system of JASCO-PU-980. The instrument incorporates an on-line organic modifier addition facility to the supercritical fluid mobile phase. The rate of flow of carbon dioxide and the modifier could be changed from 0.01-10 ml/min. The apparatus was capable of giving pressures in the range of 7.18-44.88 MPa using JASCO-880-81 back pressure regulator and temperatures in the range of 35-80°. Further the use of a variable restrictor allows for a constant flow rate of the fluid, thus producing stability in system pressure. These system improvements have made pcSFC more precise and reproducible. A JASCO-CO-975 oven provided any desired temperature between 35-80° for the column. The sample was introduced into the column using a Rheodyne-injector, model 7125 with a 20 µl external loop. Detection of the analytes was through a variable wavelength ultraviolet spectrophotometric detector UV-975 fitted with a 4 µl high pressure flow cell of 5 mm path length. A PC-based Borwin Chromatographic Software was used for data integration.

Method development:

Method development in pcSFC consists of measuring the retention times of the 4 individual drugs as a function of pressure, temperature and modifiers composition using different columns such as decyl, octadecyl, phenyl and cyano. Thereafter the optimum composition of the modifier, the pressure, temperature and the column, flow rate are to be chosen from a perusal of the accumulated data. After selecting a suitable column, the modifier composition was optimised. Methanol, a good solvent for all the 4 drugs, was chosen as the modifier. However the primary modifier, methanol, alone could not give ideal separations. The peaks of guaifenesin and acetaminophen were found to be overlapping, indicating incomplete separation. The addition of 1% methylamine as secondary modifier to methanol provided the required resolution between all the 4 drugs. After the modifier composition, expressed as the v/v fraction of methanol in carbon dioxide, was optimised, further improvements in base line separation, peak shapes and resolution were obtained by proper adjustments of pressure and temperature. Thereafter mixtures of the 4 drugs were injected using the optimised chromatographic conditions which are given in Table 1. Fig. 1 shows a typical chromatograph of a mixture of the 4 drugs. Table 2 gives the chromatographic figures of merit for the 4 drugs. As can be seen from this Table, the figures obtained denote ideal chromatography.

For calibration, eight mixture concentrations were taken. The concentrations of the drugs in the mixtures are; guaifenesin : 0.5, 1.0, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0 µg/ml, acetaminophen : 0.5, 1.0, 5.0, 10.0, 25.0, 50.0, 75.0, 100.0 µg/ml, pseudoephedrine HCl : 1.5, 5.0, 10.0, 25.0, 50.0, 75.0, 100.0 µg/ml, 150.0 µg/ml and dextromethorphan HBr:1.0, 5.0, 10.0, 20.0, 25.0, 50.0, 75.0, 100.0 µg/ml.

In each case, 20 µl of the mixture solutions were injected and chromatography performed as per conditions

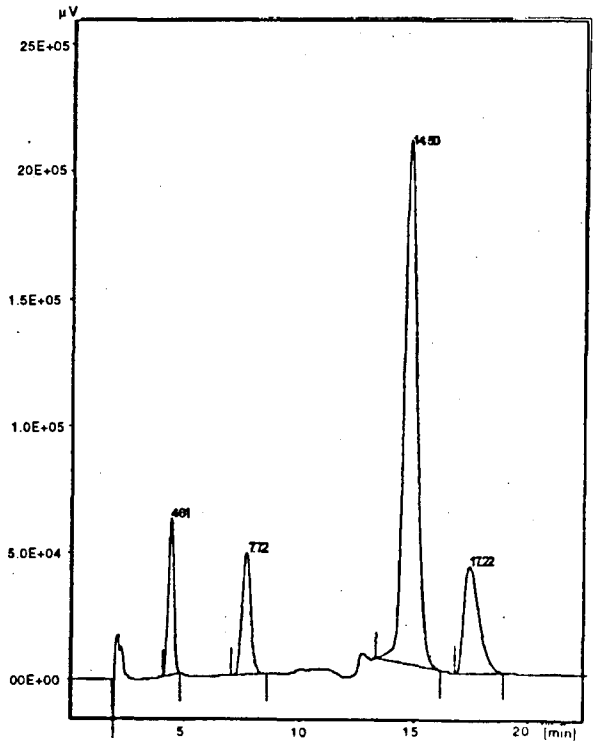

Fig. 1: Typical supercritical fluid chromatogram

Typical SFC separation of drugs eluted from a Hypersil Phenyl (250 x 4.6 mm) 5 µm column under steady state conditions. The retention times are : 1) Guaifenesin (25 µg/ml) - 4.61 min 2) Acetaminophen (25 µg/ml) - 7.72 min 3) Pseudoephedrine HCl (250 µg/ml) - 14.50 min 4) Dextromethorphan HBr (75 µg/ml) - 17.22 min.

TABLE 1: CHROMATOGRAPHIC CONDITIONS

Apparatus	:	JASCO-900 series SF Chromatograph
Column	:	Hypersil-Phenyl (250 x 4.6 mm) 5 µm
Mobile Phase	:	4.3% (v/v) of methanol (containing 1% methylamine) modified carbon dioxide.
Flow rate of CO_2	:	1.5 ml min^{-1}
Flow rate of Modifier	:	0.25 ml min^{-1}
Back Pressure	:	13.73 MPa
Temperature	:	35°
Detection	:	Spectrophotometric at 210 nm
Injection Volume	:	20 µl
Retention Time	:	
Guaifenesin	:	~ 4.61 min
Acetaminophen	:	~ 7.72 min
Pseudoephedrine HCl	:	~ 14.50 min
Dextromethorphan HBr	:	~ 17.22 min
Run Time	:	~ 18.0 min

TABLE 2: CHROMATOGRAPHIC FIGURES OF MERIT FOR PCSFC SEPARATION OF FOUR DRUGS

Drug	Guaifenesin	Acetaminophen	Pseudophedrine HCl	Dextromethorphan HBr
Retention Time (t_R, min)	4.61	7.72	14.50	17.22
Relative Retention Time (RRT)	1.0	1.75	3.37	4.01
Capacity factor (K')	1.23	2.96	6.59	8.05
Selectivity factor (α)	—	2.41	2.23	1.22
Resolution (R)	—	5.07	6.67	2.07
Tailing factor (T)	1.07	1.10	1.00	1.02
No. of Plates (N)	2432	1671	2834	2093
HETP (h, cm)	0.010	0.015	0.009	0.012

shown in Table 1. Detector responses were measured in peak heights, not in peak areas[9]. Each experiment was repeated seven times and the mean response values were used for calculations. It was found that the detector responses were rectilinear to concentrations. The data was analysed by linear regression least squares fit and the parameters are listed in Table 3.

Assay of formulation:

For the assay of tablets, twenty tablets were powdered and homogenised. A quantity of the powder equivalent to 200 mg of guaifenesin was weighed and dissolved in 100 ml of alcohol. From the filtered solution a quantity was removed and diluted ten-fold. Further dilutions were such that the concentration was contained in the linear dynamic range given in Table 3. Peak response, measured as peak heights, were related to the slope and intercept values given in Table 3 and the concentrations determined. The experiment was repeated seven times and the mean values obtained for the dosage were as follows; guaifenesin - 200.87 ± 2.11 mg; acetaminophen-325.12 ± 0.98 mg, pseudoephedrine hydrochloride 30.01 ± 0.26 mg, dextromethorphan hydrobromide 15.01 ± 0.09 mg against the labelled amount of 200, 325, 30 and 15 mg, respectively.

TABLE 3: LINEAR REGRESSION LEAST SQUARE FIT DATA FOR PCSFC ASSAY OF FOUR DRUGS

Drug	Guaifenesin	Acetaminophen	Pseudophedrine HCl	Dextromethorphan HBr
LDR (µg/ml)	0.5-50	0.5-100	1.5-250	1-100
LOD (µg/ml)	0.2	0.2	1.00	0.60
LOQ (µg/ml)	0.5	0.5	1.5	1.00
Slope (m)	426.51	526.72	178.33	122.25
Intercept (b)	-20.28	22.86	-20.52	3.108
S.D. of Slope (Sm)	3.15	0.951	0.106	0.143
S.D. of Intercept (Sb)	66.78	20.19	9.758	6.720
Regression (r)	0.999	0.999	0.999	0.999
Syx	133.61	40.42	14.44	12.12

TABLE 4: ACCURACY AND PRECISION OF THE PCSFC METHOD FOR SIMULTANEOUS ASSAY OF THE 4 DRUGS

Drug	Conc. added mg	Total Conc. mg	Conc. Found mg	Error (%)	Recovery (%)
Guaifenesin	5	205	192.11	6.288	93.71
200 mg	10	210	207.76	1.067	98.93
(n=7)	25	225	222.84	0.96	99.04
Acetaminophen	5	330	319.08	3.309	96.69
325 mg	10	335	339.12	1.230	101.23
(n=7)	50	375	372.86	0.571	99.43
Pseudoephedrine HCl	2	32	33.89	5.906	105.91
30 mg	5	35	36.98	5.657	105.66
(n=7)	10	40	41.12	2.8	102.8
Dextromethorphan HBr	1	16	15.24	1.6	95.25
15 mg	2	17	16.75	1.471	98.53
(n=7)	5	20	20.29	1.45	101.45

Precision and accuracy were estimated by assaying solutions of the dosage form spiked with mixtures containing known amounts of these 4 drugs. Each experiment was repeated 7 times and the mean recovery values together with the standard deviations are given in Table 4. As can be seen from this Table, recovery values are all between 93.71 to 105.66% and RSD values of replicate estimations do not exceed 2%.

RESULTS AND DISCUSSION

A comparison of the present pcSFC method and the HPLC method shows that pcSFC can replace the ion-pairing chromatography with simple reverse phase chromatography, at least in this case. The mobile phase flow programming can also be avoided. The run times are almost equal, showing that the throughput hr^{-1} is almost the same. One run of complete analysis of the dosage by HPLC leaves 15.83 ml of a mixture of acetonitrile and water as a waste while in pcSFC it leaves only 3.86 ml of comparatively pure methanol as waste, as both carbon dioxide and methylamine are volatile at room temperature. The elution order of the analytes is also very different in that while in HPLC pseudoephedrine HCl elutes out first, in pcSFC it elutes out third. It is guaifenesin which elutes first. In both the cases acetaminophen is the second and dextromethorphan HBr the last. Such a

difference in elution behaviour can be attributed to the differences in ion-pair and reverse phase chromatography.

A simple, sensitive method has been developed for the isocratic separation and simultaneous estimation of acetaminophen, guaifenesin, pseudoephedrine HCl and dextromethorphen HBr in bulk and pharmaceutical dosage forms by pcSFC. The method has been compared to a HPLC method from literature. It can be concluded that pcSFC has superior flexibility in method development and is a viable alternative to HPLC for this study.

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