Simultaneous HPLC Estimation of Acetaminophen, Chlopheniramine Maleate, Dextromethorphan Hydrobromide and Pseudoephedrine Hydrochloride in Tablets

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A simple and precise reversed phase HPLC method has been developed for the simultaneous estimation of acetaminophen, chlorpheniramine maleate, dextromethorphan hydrobromide and pseudoephedrine hydrochloride in tablet on a μ Bondpak phenyl bonded (30 cm × 3.9 mm) column using methanol:buffer (50:42.5, adjusted to pH 3.6 using orthophosphoric acid) as a mobile phase at a flow rate of 1.5 ml/min and detection at 214 nm. The retention times of acetaminophen, pseudoephedrine hydrochloride, chlorpheniramine maleate and dextromethorphan hydrobromide have been found to be 2.39, 4.01, 10.15 and 13.72 min, respectively and the recoveries from tablets were between 99-101%. Validation of the proposed method also been done. The method can be used for estimation of combination of these drugs in tablet.

Acetaminophen (ACE), a centrally and peripherally acting non-opioid analgesic and antipyretic drug, chlorpheniramine maleate (CPM), a potent antiallergic agent with moderate sedative action, dextromethorphan hydrobromide (DMH), a cough suppressant and antitussive agent and pseudoephedrine hydrochloride (PEH), a bronchodilator, are used in various multicomponent formulations for symptomatic treatment of common cold and cough. One such tablet formulation containing ACE (325 mg), CPM (2 mg), DMH (15 mg) and PEH (30 mg) is available in the market (Respren Tablets of Ethnor Pharmaceuticals Ltd.).

Many methods for the determination of ACE, CPM, DMH and PEH, individually as well as in combination, are reported[1-7]. However, no method for simultaneous estimation of these drugs in combined dosage form is available. The present work describes a simple, precise and accurate reversed phase HPLC method for the simultaneous estimation of these components in tablet. All chemicals/solvents used were of AR/HPLC grade. Reference standards of ACE, CPM, DMH and PEH were provided by Parke-Davis India Ltd., Hyderabad.

Waters® and Shimadzu® HPLC systems with a μ Bondpak phenyl bonded (30 cm x 3.9 mm) column as a stationary phase. Methanol and buffer (0.34 g of monobasic potassium phosphate, 0.15 g of triethylamine hydrochloride and 0.25 g of sodium lauryl sulfate in each 450 ml of solution) in the ratio of 50:42.5, pH adjusted to 3.6 with orthophosphoric acid was used as a mobile phase at a flow rate of 1.5 ml/min. Injection volume was 10 μl (Rheodyne injector) and detection was done at 214 nm. The mobile phase was filtered through a 0.45 μ membrane filter (Millipore), degassed and sonicated for 10 min. The separation was carried out at room temperature (about 20°).

A mixed stock standard solution containing ACE (13.0 mg/ml), CPM (80 μg/ml), DMH (600 μg/ml) and PEH (1.2 mg/ml) was prepared in mobile phase. A working standard solution was prepared by diluting 10.0 ml of a mixed stock standard solution to 100.0 ml with mobile phase. Sample solution was prepared by shaking tablet powder equivalent to 130 mg of ACE in the mobile phase, filtering (Whatman No. 1) and making up the volume to 100.0 ml. A steady baseline was recorded with optimized chromatographic conditions. Chromatograms of standard solution (five replicate) and sample solution (two replicate) were recorded. The concentrations of each component in sample solution were obtained by comparing with the standard solution.

Accuracy was measured using five samples (within 80-120% of label claim) with accurate concentration of components were prepared by using the stock standard solution

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and appropriately weighed quantity of synthetic mixture of the drug product components (Placebo). These were analyzed by injecting three replicate of each sample solution and the percent recovery was calculated. Precision was studied by analyzing six sample solutions (two replicate of each) prepared from homogenous sample and concentrations of drugs were calculated. Specificity was carried out by exposing the samples to different stress conditions for 24 h such as acidic (0.1N HCl, 1.0 ml, 40°), basic (0.1N NaOH, 1.0 ml, 40°), oxidation (3% v/v H_2O_2, 1.0 ml, 40°), heat (60°), UV light (260nm, 40°), and humidity (75% RH, 40°), before analysis by proposed method. Linearity and Range was demonstrated with the results of accuracy. The curve was plotted using percent label claim Vs area under curve. Ruggedness of the method was evaluated by carrying out the experiment on different instruments (Waters and Shimadzu), by different analysts and on different days. Stability of sample solution was ascertained by analyzing it periodically.

The chromatographic parameters were validated by system suitability studies and resolution, peak asymmetry and column efficiency were determined (Table 1). Accuracy studies indicated recoveries of the drugs between 99-101% (Table 2). The precision data showed the repeatability of the assay procedure as satisfactory (Table 2). The results of specificity studies indicated no interference from other active ingredients, excipients, impurities and degradation products due to various stress conditions, and assured that the peak response was due to a single component only. For all the four drugs in formulation, percent label claim Vs area under curve plots show a linear relationship with correlation coefficient more than 0.99. Ruggedness study signified the reproducibility of the method under different conditions (instruments, days and analysts). Solution stability studies shows that the sample solution is stable for 24 h. All four drugs estimated within 15 min, hence the present method is cost effective and faster, can be used for the routine analysis of these drugs from tablet formulation.

TABLE 1: SYSTEM SUITABILITY PARAMETERS

Parameters	ACE	PEH	CPM	DMH
Resolution	4.8	10.5	13.6	4.1
Capacity factor	1.6	3.4	10.6	14.6
Tailing Factor	1.3	1.4	1.2	1.3
Theoretical Plates (Per meter)	9623	11306	9733	11506

TABLE 2: ESTIMATION OF TABLET FORMULATION

Drugs	Label Claim (mg/tablet)	Estimated*		% Recovery**
		mg/tablet	% Label Claim	
ACE	325	323.6	99.6(0.8)	99.9(0.4)
PEH	30	29.6	98.5(1.0)	99.8(0.9)
CPM	2	2.0	101.5(0.7)	99.7(0.3)
DMH	15	14.9	99.7(0.7)	100.2(0.7)

* Mean (%RSD) of six observations. ** Mean (%RSD) of five observations. Assay and Precision was studied by analyzing six sample solutions (two replicate of each) prepared from homogenous sample. Accuracy (Recovery) was measured using five samples with accurate concentration of components (80-120% of L.C.) and appropriately weighed quantity of synthetic mixture of the drug product components (Placebo).

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Synthesis and Antimicrobial Activity of Some New 1,2,4-Triazoles

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A variety of 1,2,4-triazole derivatives were synthesized by esterifying substituted aryl carboxylic acid separately. It was then treated with hydrazine hydrate to yield corresponding aromatic acid hydrazides. When these hydrazides were treated with KOH and CS_2, oxadiazoles were obtained. Thus obtained oxadiazoles on further treatment with different acid hydrazides afforded three different series of 1,2,4-triazole derivatives. All the newly synthesized compounds were screened for their antimicrobial activities and 1,2,4-triazoles of anthranilic acid series were found to be very active than the other two series of compounds.

Literature revealed that 1,2,4-triazoles[1,2] showed significant antimicrobial activities and their derivatives[3,4] were also known for their interesting antitubercular and pharmacological activities. This observation and our interest in the synthesis of biologically active heterocyclic compounds encouraged us to synthesis 1,2,4 -triazoles from aryl substituted carboxylic acid by using different acid hydrazides such as hydrazine hydrate, vitamin. nicotinamide and the antitubercular agents isoniazid, pyrazinamide and ethionamide to give antimicrobial activities in triazole form.

The three different triazole series 4A a-e, 4B a-e and 4C a-e were conveniently synthesized by acid esterification of substituted aryl carboxylic acid with ethanol/concentrated Hcl to yield 1A, 1B and 1C which upon refluxing with hydrazine hydrate yield corresponding aromatic acid hydrazides 2A, 2B and 2C. These acid hydrazides 2A, 2B and 2C on treatment with KOH and CS_2 yielded corresponding 3A, 3B and 3C oxadiazoles The title compounds 1,2,4-triazoles were prepared by refluxing oxadiazoles of 3A, 3B and 3C with different acid hydrazides as shown in the scheme. All the newly synthesized compounds were screened for their antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*; and antifungal activity against *Candida albicans* using Kirby Bauer method.

Melting points were determined in open capillary tubes and are uncorrected. Precoated TLC plates were used to check the purity of the compounds. IR spectra were recorded using KBr pellets on a Jasco IR Spectrometer and ^1H NMR spectra were recorded on a Bruker AC 300 MHz FTNMR using TMS as internal standard and chemical shifts were expressed in δ ppm. Elemental analysis was carried out using a Perkin Elmer –2400 CHN analyzer. The compounds 1A, 1B and 1C, 2A, 2B and 2C, 3A, 3B and 3C were pre-

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