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A Validated RP-HPLC Method for Simultaneous Estimation of Acetaminophen and Methocarbamol in Tablets

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A rapid, sensitive and specific RP-HPLC method involving UV detection was developed and validated for simultaneous determination and quantification of acetaminophen and methocarbamol. Chromatography was carried out on a pre-packed develosil-C18 (5 μm 25 × 0.46 cm) column using water: methanol:glacial acetic acid (60:40:1.5) as mobile phase at a flow rate of 1 mL min^{-1} and effluent were monitored at 273 nm. The assay was linear over the concentration range of 62.5-500 μg mL^{-1} and 50-400 μg mL^{-1} for acetaminophen and methocarbamol, respectively. The per cent recovery was ranging from 99.98-100.03, 99.75-100.07 % for acetaminophen and methocarbamol, respectively with an intra day, inter-day precision RSD of 0.678, 0.487 and 0.607, 0.563 for acetaminophen and methocarbamol. The method does require only 7 min as run time for analysis which prove the adoptability of the method for the routine quality control of these drugs.

Key Words: HPLC, Acetaminophen, Methocarbamol.

INTRODUCTION

Acetaminophen[1,2] chemically N-(4-hydroxyphenyl)ethanamide, is used in the symptomatic treatment of pain and fever. Methocarbamol is chemically 2-hydroxy-3-(2-methoxyphenoxy)-propylamino formate, is used in the treatment of muscle spasm. Few methods[3-9] have been reported for the estimation of acetaminophen and methocarbamol by HPLC method. Only one method has been reported for the simultaneous estimation of acetaminophen and methocarbamol in tablets by HPLC[10] but the method takes long runtime and the peaks eluted were broadened. The present paper describes a validated HPLC with low runtime reduced peak tailing for the estimation of acetaminophen and methocarbamol.

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EXPERIMENTAL

Working standards of acetaminophen and methocarbamol were obtained from well reputed research laboratories. The purities of these standards were 99.57 and 99.32 %, respectively. HPLC grade methanol, HPLC grade glacial acetic acid and Milli Q Water are procured from the market. The separation was carried out on isocratic HPLC system (Waters) with Empower software with RP Develosil C-18 column (100 × 4.6 mm, 5 µm) mobile phase is a filtered and degassed mixture of water, methanol and glacial acetic acid was prepared in the ratio of 60:40:1.5.

Standard preparation: About 50 mg of acetaminophen and 40 mg of methocarbamol were accurately weighed and transferred to a 200 mL volumetric flask the mixture was then dissolved in diluent by sonication to give standard stock solution of 0.25 mg and 0.2 mg mL^{-1}, acetaminophen and methocarbamol respectively.

Chromatographic conditions: Flowrate 1.0 mL/min; detection wavelength 273 nm; injection volume: 10 µL; column used: develosil C-18 column (100 × 4.6 × 5 µm); column temperature: 25 ºC. Mobile phase:water:methanol: glacial acetic acid; diluent:water:methanol (60:40).

Method development: Working standards of various concentrations were prepared by taking aliquots of standard solution and diluted to get required concentrations for calibration plot and which were injected.

Assay preparation for commercial formulations: Twenty tablets were weighed accurately and finely powdered. Powder equivalent to 500 mg of acetaminophen and 400 mg of methocarbamol was transferred into 200 mL volumetric flask and dissolved in sufficient amount of diluent and sonicated to dissolve. Solution was filtered through 0.45 µ membrane filter and then the filtrate was further diluted to get the required concentrations.

Procedure: 10 µL of the standard preparation and assay preparation were separately injected and chromatographed.

Method validation

Method linearity: Linearity was demonstrated by analyzing eight different concentrations of active compound. Peak areas were recorded for all the peaks and calibration plot was constructed by plotting peak area *vs.* concentration of acetaminophen and methocarbamol which were found to be linear in the range of 62.5 to 500 µg mL^{-1} and 50 to 400 µg mL^{-1} for acetaminophen and methocarbamol, respectively. Coefficient of correlation for acetaminophen was 0.9998 and for methocarbamol was 0.9999 (Fig. 1).

Accuracy: Accuracy was done by recovery study using standard addition method, known amount of standard acetaminophen and methocarbamol was added in to pre-analyzed samples and subjected to proposed HPLC method. The results of recovery studies are shown in Table-1.

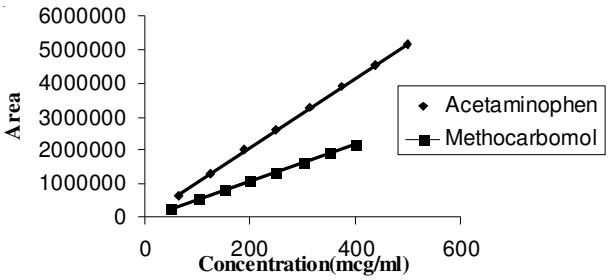

Fig. 1. Linearity graph of acetaminophen and methocarbomol

TABLE-1
ANALYSIS OF TABLET CONTAINING ACETAMINOPHEN AND METHOCARBAMOL

Formulation	Drug	Label claim	Amount found (mg)*	Found (%)	Amount std. added	Amount recovered	Recovery (%)
Tablet	Acetaminophen	500	500.05	100.01	1	0.9987	99.87
	Methocarbamol	400	399.89	99.97	1	1.0150	101.57

*Mean of six determination (n = 6).

Method precision: To demonstrate agreement among results, a series of measurements are done with acetaminophen and methocarbamol, six replicate injections of the specific standard at various time intervals on the same day were injected in to the chromatograph and the value of % RSD was found to be 0.678 and 0.487 for acetaminophen and methocarbamol, respectively. In inter-day precision same standard was injected on different days and the found % RSD were 0.607, 0.563 for acetaminophen and methocarbamol respectively (Table-2).

TABLE-2
INTER AND INTRA-DAY PRECISION STUDIES

Amount found on	Amount found on			
	Intra-day		Inter-day	
	Mean (n = 6)	RSD (%)	Mean (n = 6)	RSD (%)
Acetaminophen (250 µg/moL)	250.07	0.678	249.87	0.607
Methocarbamol (500 µg/moL)	499.56	0.487	499.44	0.563

RESULTS AND DISCUSSION

The regression value was found to be 0.9998 for acetaminophen and 0.9999 for methocarbamol, which shows the response is linear from 62.5 to 500 µg mL^{-1} for acetaminophen and from 50 to 400 µg mL^{-1} for methocarbamol. Selectivity experiment showed that there is no interference or

overlapping of the peaks either due to excipients or diluents with the main peak of acetaminophen and methocarbamol. High percentage of recovery shows that the method is free from interference of acetaminophen from methocarbamol and *vice-versa*. The % RSD for precision is < 2 which confirms that method is sufficiently precise and the total run time required for the method is only 7 min for eluting acetaminophen and methocarbamol, respectively. The proposed method is simple, fast, accurate, precise and can be used for routine analysis in quality control of acetaminophen and methocarbamol.

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REFERENCES

1. The Merck Index, Merck research Laboratories, Division of Merck & Co. INC, White House Station, edn. 13, p. 10 & 1070 (2004).
2. E.F.J. Reynolds, Martindale: The Extra Pharmacopoeia, Royal Pharmaceutical Society, London, edn. 32, p. 76, 1395 (1999).
3. D. Bose, A. Durgbanshi, A. Martinavarao, D. Capella, M.E. Peiro and S.C. Broch, *J. Chromatogr. Sci.*, **43**, 313 (2005).
4. J. Parojcic, K. Karljikovic-Rajic, Z. Duric, M. Jovanovic and S. Ibric, *Biopharm. Drug Dispos.*, **24**, 309 (2003).
5. H. Fujino, H. Yoshida, H. Nohta and M. Yamaguchi, *Japan Soc. Anal. Chem. Anal. Sci.*, **21**, 1121 (2005).
6. R. Nageswara Rao and A. Narasaraju, *Japan Soc. Anal. Chem. Anal. Sci.*, **22**, 287 (2006).
7. J. Weng, N. Weng, J.W. Lee and J.D. Hulse, *Chromatogr. B Biomed. Appl.*, **654**, 287 (1994).
8. H. Farsan, Soruie and N.J. Gharavi, *Pharm. Pharmacol.*, **51**, 873 (1999).
9. M.R. Koupai-Abyazani, B. Esaw and B. Laviolette, *J. Anal. Toxicol.*, **21**, 301 (1997).
10. N. Erk, *J. Pharm. Biomed. Anal.*, **24**, 469 (2001).

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