Determination of Methylene Chloride Organic Volatile Impurity in Marketed Formulations of Ciprofloxacin, Norfloxacin, Pefloxacin and Ofloxacin

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A simple and sensitive method for the determination of methylene chloride as residual solvent was developed and validated on gas liquid chromatograph fitted with flame ionization detector. The carrier gas was nitrogen, and separation was carried out on BP 5 capillary column consisting of 5% phenyl and 95% dimethyl polysiloxane stationary phase. The retention time for methylene chloride was 5.4 min. The method was extended for determination of the methylene chloride organic volatile impurity in the marketed formulations of ciprofloxacin hydrochloride, norfloxacin, pefloxacin and ofloxacin.

Organic solvents are entrapped within the formulation either during the course of manufacture of active pharmaceutical ingredients or during the coating of the formulation. These solvents are used frequently to dissolve film-coating materials to facilitate application onto compressed tablets. These tablets are subjected to air-drying to remove all the organic solvents from the coat of finished product. The residual levels of these organic solvents in the tablet cores and film coats are critical, as beyond permissible limits, they are likely to cause undesirable side effects or alter some kind of physicochemical property of the active pharmaceutical ingredient. Hence it becomes necessary to limit the amount of these residual solvents, which can be called organic volatile impurities to certain levels within the ICH-prescribed limits. The most sensitive among the methods for monitoring the amount of residual solvent in the marketed solid dosage formulations is the gas chromatographic method. Literature survey on residual solvent testing in active pharmaceutical ingredients and coated tablets cited gas chromatographic methods for the determination of organic volatile impurities[1-10].

The objective of the present study was to develop a method for identification and quantification of methylene

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chloride by direct injection capillary gas chromatographic method and apply the same for its determination in marketed formulations using nitrogen as the carrier gas. Methylene chloride is used in the basic drug manufacture as a solvent, and more precisely, in coating process. A gas chromatograph GC-17A Version-3, equipped with flame ionization detector (FID), was used for the study. A general-purpose capillary column BP-5 (SGE) with composition of 5% phenyl and 95% dimethyl polysiloxane with 0.25 mm internal diameter, 30 m length, and film thickness of 0.25 mm was used. Analytical grade methylene chloride (Qualigens, Mumbai) and dimethyl sulfoxide (Thomas Baker, Mumbai) were used.

In the proposed method, the following working instrumental variables were enabled on the gas chromatograph. The injection port temperature was 250° and detector temperature was 270°. The flow rate of carrier gas was 1.0 ml/min with control mode of split of ratio 1:2. The column pressure was initially maintained at 30 kpa. A temperature programme was devised with the initial increase in temperature of 40° up to 5 min; thereafter, the temperature was increased up to 60° in 5 min (at the rate of 4°/min), 100° in 5 min (at the rate of 8°/min), and 260° in 8 min (at the rate of 20°/min) to prevent the interference of dimethyl sulfoxide solvent with methylene chloride.

In a 10 ml volumetric flask, standard stock solution was prepared by diluting 1 ml (1.307 gm) of methylene chloride to 10 ml with dimethyl sulfoxide. Working standard solution was prepared from the stock solution by diluting 0.1 ml of the stock to 10 ml with dimethyl sulfoxide. With a Hamilton syringe, volumes of 1, 2, 3, 4, and 5 µl of working standards were injected into gas chromatograph. A retention time of 5.4 min was recorded for methylene chloride. The linearity was determined by a series of three replicate injections of standards. The evaluation was made by visual inspection of a plot of signal height or peak area as a function of analyte concentration. The data of peak areas were obtained from the chromatograms for methylene chloride, and the linearity curves were plotted. Further, the method was validated for parameters like accuracy, precision, robustness, and ruggedness.

Linearity was obtained in the concentration range of 1.3 to 5.2 µg. Linearity coefficient was 0.99, and the percent curve fitting was found to be 99.99. Limit of detection was 0.33 µg. Precision of the method was determined by replicate injections. % RSD was found to be 2.62, which was within the limits of 15% as specified by USP. Specificity of the method was found out through non-interference of the blank, dimethyl sulfoxide, by performing the analysis of the blank in identical conditions of the method. Absence of peaks in the retention time up to 15 min indicated specificity for the proposed method. Accuracy of the method was determined through recovery studies of the organic volatile impurity of methylene chloride by adding standard substance to previously analysed sample formulation at three levels. Percent recovery of the impurity was found to be in the range of 98-99.4. Robustness was determined by carrying out the determination during which the temperature programming was slightly altered within 10% of the values. Percent recovery was found to be 99.3-103.7. The low values of the RSD with small variations in the temperature programming indicated the lack of influence on test results by operational and environmental variables for the proposed method. Ruggedness was determined by performing the same method by different analysts on different days to check the reproducibility. The test result was found to be within the limits of percent recovery of 95-100. Theoretical plates per

TABLE 1: AMOUNT OF METHYLENE CHLORIDE ORGANIC VOLATILE IMPURITY PRESENT IN MARKETED FORMULATIONS

Sample	Brand name	Manufacturer	Dose(mg)	Amt. of methylene chloride per tablet* (mg)	% RSD
Ciprofloxacin	Ciflafin	Alpine Lab	500	0.00140	0.75
	Ciprolet DS	Dr. Reddy's Lab	500	0.00045	1.23
	Procip	Himanshu Pharm	500	0.00053	1.79
Norfloxacin	Norbid	Alembic	400	0.00090	0.88
	Uroflox	Vista	400	0.00060	0.71
	Norflox	Cipla	400	0.00200	0.05
Pefloxacin	Pelox	Wockhardt	400	0.00043	1.89
	Perti	Dr. Reddy's Lab	400	0.00150	1.13
	Pefbid	Alembic	400	0.00120	1.64
Ofloxacin	Oflin	Zydus Cadila	200	0.00000	0.00
	Ofxin	Medley Pharm	200	0.00070	0.02
	Ofloren	Indoco Rem	200	0.00310	1.95

*Average of six determinations

column were calculated by statistical analysis to determine system suitability of the method. The number of theoretical plates per column was 22961 and the symmetry factor was 1.01.

The validated method was applied for determination of residual solvents in certain film-coated marketed antibiotic formulation of ciprofloxacin, norfloxacin, pefloxacin, and ofloxacin. Twenty tablets each containing 500 mg, 400 mg, 400 mg, and 200 mg of ciprofloxacin, norfloxacin, pefloxacin, and ofloxacin, respectively were accurately weighed separately. The tablets were crushed in a separate glass mortar into a fine powder, transferred into a stoppered conical flask, and extracted with 100 ml portion of dimethyl sulfoxide. The extract was filtered with Whatman filter paper No. 1 into a clean, dry 100 ml volumetric flask to get sample stock solution. A volume of 2 ml of the sample stock solution was diluted to 10 ml using dimethyl sulfoxide. From the filtered solution, 5 µl was injected into the gas chromatograph. Methylene chloride peak was detected at 5.4 min in the chromatograms of sample ciprofloxacin, norfloxacin, pefloxacin, and ofloxacin. Based on the peak areas recorded in the chromatograms, the amounts of organic volatile impurity present in the samples were calculated. Methylene chloride was found to be in the concentration range of 0.001 to 0.003 mg per tablet, which was well within the permissible limit of 600 ppm as required by the specification of ICH. The amounts of residual solvents found in the marketed formulations have been presented in Table 1.

REFERENCES

1. Rastogi, S.C., **Chromatographia**., 1993, 37, 211.
2. Kevin, J.M. and Thomas, W.B., **J. Chrom. Biomed. Appl**.,1996, 686, 85.
3. Hymer, B.C., **J. Chrom.**, 1988, 438, 103.
4. Hong, L. and Altorfer, H., **Chromatograpia.**, 2001, 53, 76.
5. Russo, M.V., **Chromatographia**., 1994, 39, 645.
6. Muligan, K.J. and Mc Cauley, H., **J. Chrom. Sci**., 1995, 33, 49.
7. Smith, I.D. and Water, D.G., **Analyst**., 1991, 116, 1327.
8. Capitani, F., Nocilli, F. and Porretta, G.C., **Farmaco.**, 1988, 43, 189.
9. Witschi, C. and Doelker, E., **Eur. J. Pharm. Biopharm**., 1997, 43, 215.
10. Kumar, N. and Egoville, J.C., **J. Chrom. A**., 1999, 859, 113.

Accepted 29 May 2006
Revised 8 July 2005
Received 24 January 2005
Indian J. Pharm. Sci., 2006, 68 (3): 368-370