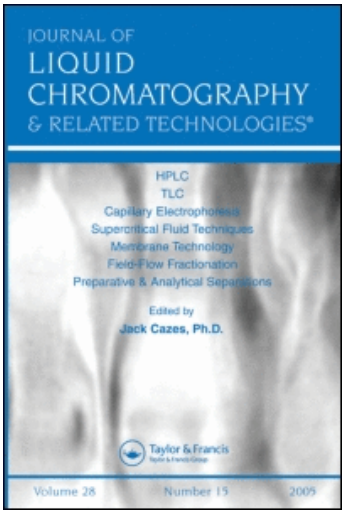

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Simultaneous Determination of Metformin in Its Multicomponent Dosage Forms with Glipizide and Gliclazide Using Micellar Liquid Chromatography

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ABSTRACT

An HPLC procedure for the simultaneous determination of metformin in combination with glipizide and gliclazide in pharmaceutical preparations is described. A Zorbax XDB C_{18} 15 cm analytical column and UV detection at wavelength 226 nm were used. In this study, various mobile phase variables were studied to determine the effects that each had on metformin, glipizide, and gliclazide. The reproducibilities in combination I for metformin and glipizide were 1.39%, 1.30%, and in combination II for metformin and gliclazide were 1.28%, 1.75%, respectively.

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The determinations of glipizide, metformin (combination I), and gliclazide, metformin (combination II) gave recoveries with respect to the values declared by the manufacturers for glipizide and metformin (combination-I) in the range 96.1–103.4%, 95.0–100.0% using peak areas, and 96.4–103.6%, 97.3–102.5% using peak heights, respectively. The recoveries for the gliclazide and metformin (combination II) were in the range 94.7–100.8%, 96.2–101.2% using peak areas, and 94.3–101.5%, 97.8–102.5% using peak heights, respectively.

Key Words: Metformin; Glipizide; Gliclazide.

INTRODUCTION

Metformin HCl is chemically 1,1-dimethyl biguanide hydrochloride. Glipizide is 1 cyclohexyl-3-[P-[2-(5-methylpyrazine-carboxamido)ethyl]-phenyl] sulfonyl urea. Gliclazide is chemically 1-(3-azabicyclo[3.3.0]oct-3-yl)-3-(*p*-tolylsulphonyl)-urea. These three drugs are oral hypoglacemic agents. A combination of 500 mg of metformin and 5 mg of glipizide (combination I), 500 mg of metformin and 80 mg of gliclazide (combination II), are available commercially as tablets.[1] These two combinations are used in the treatment of non insulin dependent diabetes mellitus (NIDDM).

A literature survey reveals that several methods are available for the estimation of metformin, glipizide, and gliclazide, individually.[2–9] But there is only one method[10] for the simultaneous estimation of these two combinations (I and II) in which an ion pair liquid chromatographic technique has been used. In the present research paper, an attempt has been made to develop a method for the simultaneous estimation of these two combinations (I and II) by using micellar chromatography.

The separation of analytes and their quantitation in multicomponent dosage forms has become a very important part of analytical chemistry. Sometimes it becomes very difficult to separate and quantitate the drugs in the multicomponent dosages by conventional analytical methodologies. Therefore, new analytical strategies and techniques are required to separate and quantitate the drugs in the multicomponent dosages of pharmaceutical preparations.

Several separation schemes have been shown to be useful for separating complex molecules and include HPLC, capillary zone electrophoresis, and to a much lesser extent, gas chromatography and supercritical fluid chromatography. Problems have been associated with these separation techniques and, although, each holds promise, none have been found to be acceptable for the routine analysis for all types of complex molecules. An alternative to these analytical techniques would be micellar HPLC (MLC).

Micellar liquid chromatography (MLC) is a technique where a micellar agent is added to a mobile phase that contains a buffer and a small amount of organic modifier. Several advantages are apparent with MLC when compared to reversed phase liquid chromatography (RPLC) Micellar liquid chromatography uses a much lower amount of organic modifier and is therefore less toxic, and gradient MLC is done without the need for long column re-equilibration.

In 1980, Armstrong and Henry first demonstrated that aqueous micellar solutions could be used as a mobile phase in reverse phase liquid chromatography (RPLC). They called this technique pseudophase or MLC.

Micellar mobile phases have certain advantages over traditional hydro-organic mobile phases in RPLC, e.g., direct injection of biologicals, resolution of optical isomers via chiral micelles, and unusual selectivity to name a few. However, there is a problem with MLC, it tends to be less efficient than conventional RPLC.

Dorsey et al.[11] were the first to address this problem. They believed the reduction in column efficiency was due to slow mass transfer, which arises principally from poor wetting of the stationary phase. Dorsey demonstrated that chromatographic efficiency in MLC can be improved by adding a small amount of propanol, 3% by volume to the mobile phase. Yarmchuck and Cline-Love[12] on the other hand, attributed the reduced efficiency associated with ionic micellar mobile phases to poor mass transfer between the micelle and the stationary phase, with the micelle exit rate constant being the limiting factor for hydrophobic solutes. Borgerding and Hinze[13] concluded that poor mass transfer within the stationary phase itself, resulting from adsorption of surfactant onto the alkyl bonded phase, is responsible for the low efficiencies observed in MLC. They demonstrated that addition of an alcohol, such as isopropanol (IPA), to a nonionic micellar solution reduces the amount of surfactant adsorbed on the stationary phase, resulting in a more efficient separation. In contrast to what has been reported by other workers, Cassidy[14] in a recent study on band broadening in MLC concluded that improvement in solute mass transfer, which can occur upon addition of propanol to an SDS micellar solution is due to changes in the structure of the micelles and not mass transfer effects related to the loading of surfactant on the bonded phase.

Several interesting separations have been accomplished using MLC. Cline Love and co-workers[15–17] reported the direct injection of serum and urine into a reversed phase column with no protein precipitation or pressure build up problems. This method was used for therapeutic drug monitoring without the requirement of sample cleanup prior to injection. Micellar liquid chromatography has been shown to be useful for the separation of amino acids and peptides[18] and proteins.[19] One study found that small changes in the concentration of surfactant produced tremendous changes in the retention of different proteins.[19] Micellar liquid chromatography has been applied for the

estimation of diuretics,[20,21] anabolic steroids,[22] and catecholmines,[23] theophylline[24] in pharmaceuticals and for the analysis of ampicillin, cloxacillin, and their related substances in pharmaceuticals.[25]

The purpose of this research was to determine the effects that each mobile phase variable had on the retention and resolution of metformin, glipizide, and gliclazide using MLC. The mobile phase variables that were studied include, the concentration of micellar agent, mobile phase ionic strength, concentration of organic modifier, and mobile phase pH. The results from these studies are discussed. From these studies, the mobile phase, which gives adequate retention times and separation of metformin, glipizide, and gliclazide, was selected for the analysis of these two combinations (I and II).

EXPERIMENTAL

Apparatus

The instrumentation consisted of Thermo separation products constametric 3500 pump, Thermo separation products AS 3000 autosampler, Thermo separation products UV 1000 detector. Data acquisition was made with PC 1000 software version 3.5.1. The Zorbax XDB C_{18} column (4.6×150 mm, 5 μm) was used for the analysis. The mobile phase flow rate was 1.2 mL/min. The detection was performed in UV at 226 nm. All the experiments were carried out at a temperature of 30°C.

Reagents and Chemicals

Disodium hydrogen phosphate, Ortho-phosphoric acid (A.R. grade), Isopropyl alcohol, and acetonitrile (HPLC grade) were obtained from Qualigens Fine Chemicals, Dr. Annie Besant Road, Mumbai, Maharashtra State, India.

Sodium dodecyl sulphate (SDS) was obtained from E. Merck (India) Limited, Worli, Mumbai. Glipizide, Gliclazide, and metformin standards were obtained from Wockhardt Research Centre, Aurangabad, Maharashtra State, India. The tablets of combination I and combination II were purchased from the market. Nylon membrane filters (0.45 μm) were obtained from (Advanced Microdevices Pvt. Ltd. 21, Industrial Area, Ambala Cantt, India).

Whatman 41 filter paper was obtained from Whatman International Ltd., Maidstone, England.

Double distilled water was used throughout the procedure. The micellar mobile phase was prepared by using 2.5 mM disodium hydrogen phosphate and 50 mM SDS and 5% IPA, pH adjusted to 7.2 with 10% ortho-phosphoric acid. The mobile phase was vacuum filtered through 0.45 μm nylon membranes.

Diluents for standard and sample preparations was prepared consisting of 10 mM disodium hydrogen phosphate (pH 9.3) and acetonitrile in the ratio of 50 : 50 (v/v).

Standard Preparations

Stock standard solutions of metformin (5 mg/mL), glipizide (1 mg/mL), and gliclazide (0.8 mg/mL) was first prepared in a diluent. The solutions were sonicated for 5 min to dissolve the standards. The stock standard solution of glipizide was further diluted in the mobile phase to obtain (0.05 mg/mL). Working standard solutions for calibration curves were prepared by the subsequent dilution of the stock standard solutions with mobile phase.

Sample Preparations

For the analysis of tablets, 10 tablets were weighed and finely ground in a mortar. For combination I the portion equivalent to 5 mg of glipizide and 500 mg of metformin was transferred in a 100 mL volumetric flask, 50 mL of diluent was then added, and sonication was done for 15 min with swirling. After sonication, the volume was made up to the mark with the diluent, and mixed well. The solution was filtered through Whatman filter paper 41. The first 5 mL portion of the filtrate was rejected and then 2 mL of the filtered solution was transferred into a 50 mL volumetric flask and diluted with the mobile phase.

For combination II, the portion equivalent to 40 mg of gliclazide and 250 mg of metformin was taken and transferred in 100 mL volumetric flask, 50 mL of diluent was then added, and sonication was done for 15 min with swirling. After sonication, it was made up to volume with the diluent, and mixed well. The solution was filtered through Whatman filter paper 41. The first 5 mL portion of the filtrate was rejected and then 2 mL of the filtered solution was transferred into a 25 mL volumetric flask and diluted with the mobile phase. For both the combinations, six determinations were performed.

RESULTS AND DISCUSSION

Micellar liquid chromatography mobile phases consist of a surfactant, a buffer, and a low concentration of organic modifier. A major advantage of MLC is that the mobile phases contain a much lower concentration of organic modifier than a reversed phase system and is, therefore, less toxic. The surfactants used in MLC consist of two portions that contain distinctly different properties, a polar head group and a hydrocarbon tail. These properties allow

the surfactant to adsorb at interfaces (stationary phase) where both the hydrophobic and hydrophilic character can be satisfied. The formation of micelles is the result of opposing forces, hydrophilic and hydrophobic. When the critical micelle concentration is achieved, the surfactant molecules arrange in such a way that the hydrophobic tails are oriented towards the centre of the aggregate and the polar heads point outwards.[26] The repulsion between the polar head groups is the controlling force that determines the size and shape of the micelles.

The separation mechanism in MLC is similar to RPLC, in that the primary equilibrium of the analyte is between the mobile phase and the stationary phase. In MLC a secondary equilibrium is also involved in the separation, this equilibrium is the partitioning of the analyte between the mobile phase and the micelles.[26,27]

Various mobile phase parameters will have an effect on the retention and separation of organic analytes. The parameters that were studied include concentration of surfactant, ionic strength of mobile phase, concentration of organic modifier, and mobile phase pH.

Effect of Ionic Strength of Mobile Phase

In MLC, electrostatic interactions are involved between a charged analyte and the micelle in the diffuse secondary layer, while hydrophobic interactions take place in the hydrophobic inner portion of the micelle. Armstrong and Stine[28] have shown that the thickness of the double layer decreases with increasing ionic strength, which allows hydrophobic interactions to take place between the analyte and the micelle. Anti-binding analytes (compounds that are strongly excluded or repelled from a micelle) have been found to have increased retention with higher ionic strength mobile phases.[32] For the transition from anti-binding to non-binding to binding to occur, the analyte ion must have enough hydrophobic character to associate with the nonpolar portion of the micelle, overcoming electrostatic repulsion. Bromophenol blue has been shown to change an anti-binding to a binding analyte with a corresponding increase in retention, using SDS in mobile phase with 0.02 M NaCl added.[28]

Figure 1 shows how the ionic strength affects the retention of metformin, glipizide, and gliclazide. It was observed that when the ionic strength increased in the mobile phase, metformin showed reduction in retention time, whereas glipizide and gliclazide were not affected. The reduction in retention time for metformin may be because of its binding characteristics. The lack of effect on the retentions of glipizide and gliclazide could be attributed to their non-binding character.

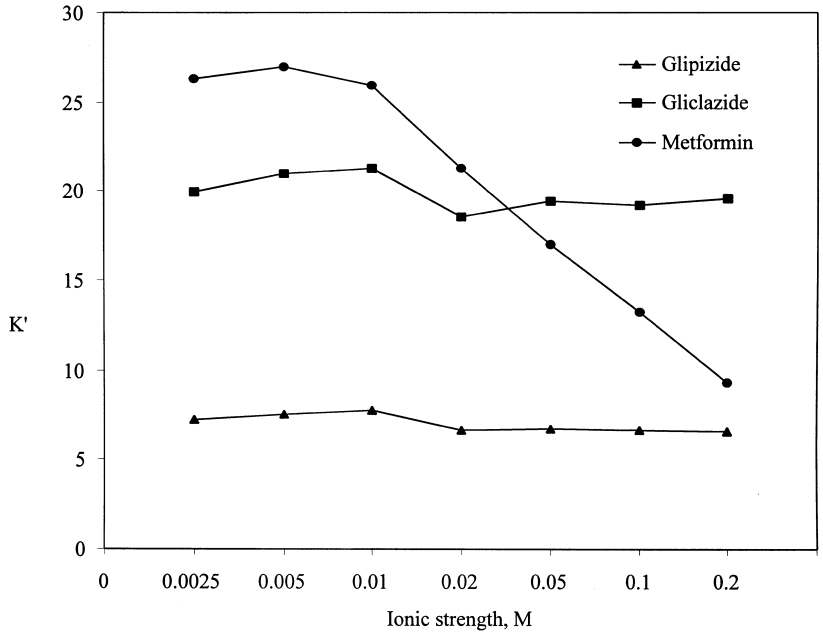

Figure 1. Effect of ionic strength on the retention of glipizide, gliclazide, and metformin. Mobile phase: 2.5 mM disodium hydrogen phosphate, 50 mM SDS, 5% IPA, pH 7.2.

Effect of Micellar Concentration

When the concentration of a micellar agent was increased in the mobile phase, a corresponding decrease in analyte retention was usually observed.[29] The rate at which the retention of the analyte changes varies with the charge and hydrophobicity of solutes, as well as, the length of the alkyl chain, charge, and concentration of the micelles.[30] A study done by Bailey and Cassidy[31] showed that the efficiency of the micellar system improved for hydrophobic analytes but not for polar analytes as the micellar concentration was increased.

Figure 2 shows how the concentration of SDS influenced the retention of metformin, glipizide, and gliclazide. As the concentration of SDS was increased in mobile phase, the retention of metformin, glipizide, and gliclazide were decreased. This would be expected since at low concentration of micellar agent, the chromatographic system resembles conventional RPLC. As the concentration of micellar agent is increased the number of micelles in the system increases and binding between the analyte and the micelles increases.[32]

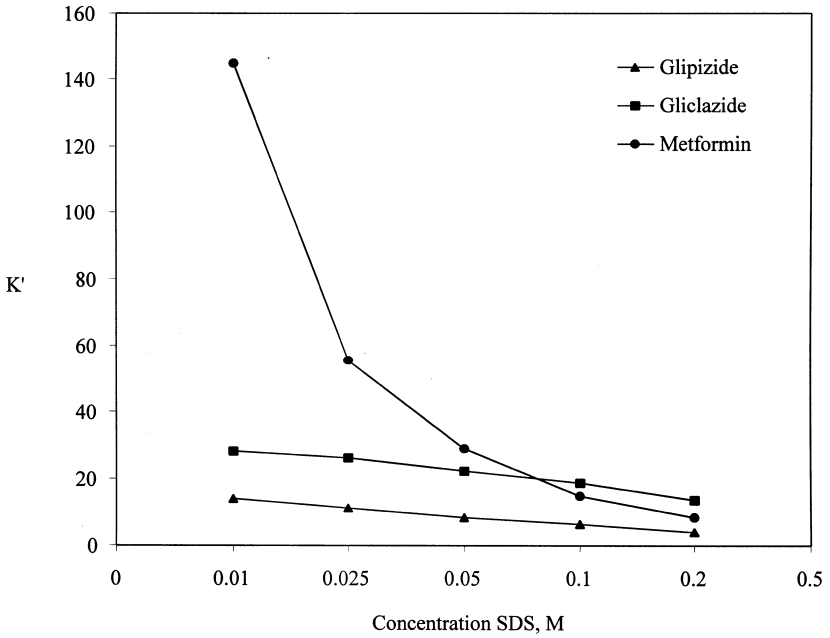

Figure 2. Effect of concentration of SDS on the retention of glipizide, gliclazide and metformin. Mobile phase: 2.5 mM disodium hydrogen phosphate, 50 mM SDS, 5% IPA, pH 7.2.

Change in elution order was observed for metformin, at and above, 0.1 M concentration of SDS and may be due to differences in the binding constants of the micelle and the analyte. Selectivity between analytes may change due to the contribution of electrostatic and hydrophobic interactions, which is dependent on the structure of the compound. From this study, it was observed that the concentration of 50 mM SDS is adequate for the retention and separation of metformin, glipizide, and gliclazide.

Effect of Mobile Phase pH

The micellar mobile phase pH will have a dramatic effect on the retention of weak organic acids and bases. Partition coefficients for the micelle-analyte interactions are different for the associated and unassociated forms. Several studies have shown that small changes in the mobile phase pH will have an effect on retention, especially when the mobile phase pH is close to the analyte's pk_a value.[33–35] Adsorption of anionic surfactant monomers on the

surface of a C_8 stationary phase cause protonated organic bases to be retained for a longer period of time than the neutral free base form, due to electrostatic attraction. Research has also shown, that the dependence of k' on pH at a constant concentration of micellar agent is sigmoidal if there is no electrostatic repulsion between any of the acid base forms and surfactant molecules.[36] It was observed that the effect of pH on retention of metformin and gliclazide is more pronounced than that on the retention of glipizide (Fig. 3).

Effect of Organic Modifier Concentration

The amount of organic modifier present in the mobile phase will have an effect on analyte retention. Khaledi and co-workers[37] have shown that elution strength increased with an increase in the organic solvent concentration. A corresponding enhancement in the separation selectivity was also observed. The selectivity enhancement was found to occur systematically, and was observed for a large number of ionic and nonionic compound with different functional groups, and also for two different surfactants, one anionic and one

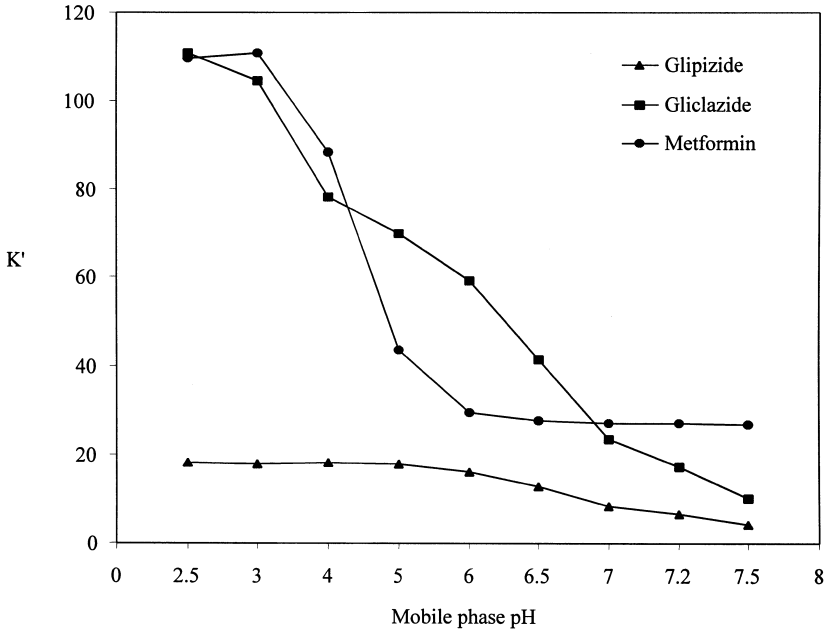

Figure 3. Effect of mobile phase pH on the retention of glipizide, gliclazide, and metformin. Mobile phase: 2.5 mM disodium hydrogen phosphate, 50 mM SDS, 5% IPA, pH 7.2.

cationic. The selectivity enhancement was credited to competing partitioning equilibria in micellar HPLC systems and/or to the characteristics of micelles to compartmentalize solutes and organic solvents.[37]

Some concern has been expressed that micellar mobile phases would act like a hydro-organic system at a higher concentration of organic modifier. This however, was shown not to be the case, it has been demonstrated that a micellar eluent that contains up to 20% IPA does not change to a hydro-organic system.[18] The addition of an organic modifier actually enhances the solvent strength and selectivity for some ionic and nonionic analytes. Retention characteristics for a solvent–water–micellar system were also found to be similar to a purely aqueous micellar eluent.[38,39] It was concluded from these studies, that the micelle influences the role of an organic modifier in the mobile phase.

Figure 4 shows the effect of IPA on the retention of metformin, glipizide, and gliclazide. When the concentration of IPA in the mobile phase was very low, up to 1.5%, retention of metformin, glipizide, and gliclazide was extremely high, but the retentions decreased with increasing concentration of IPA.

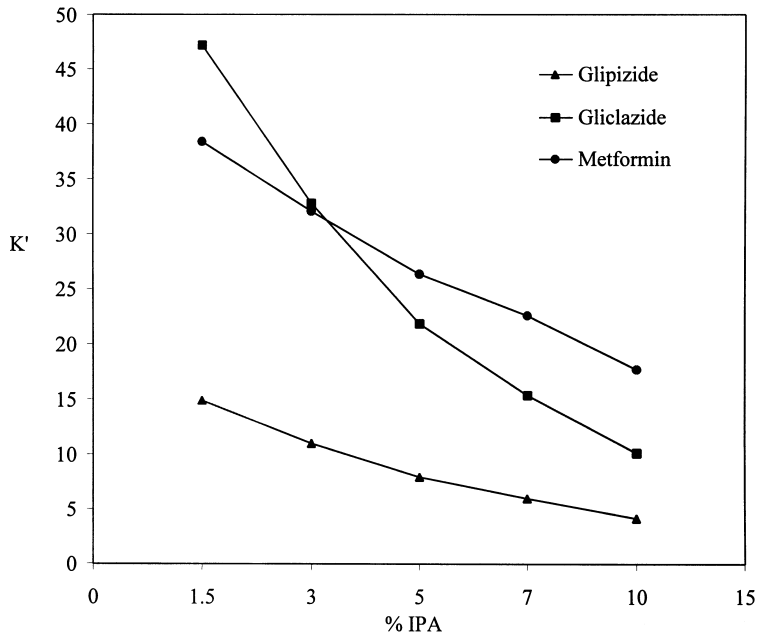

Figure 4. Effect of organic modifier concentration on retention of glipizide, gliclazide, and metformin. Mobile phase: 2.5 mM disodium hydrogen phosphate, 50 mM SDS, 5% IPA, pH 7.2.

Optimized Chromatography

From the above studies, by changing various variable parameters of mobile phase, it was observed that the separation of the analytes in combination I and II was accomplished with the mobile phase consisting of 50 mM SDS, 2.5 mM disodium hydrogen phosphate and 5% IPA, pH adjusted to 7.2 with 10% orthophosphoric acid. This mobile phase gave adequate retention and good resolution for the components and was, therefore, selected for further analysis of combination I and combination II.

System Suitability

The mixed standard solution for combination I was prepared containing 2 μg/mL glipizide and 200 μg/mL of metformin in mobile phase. Similarly, for combination II, a mixed standard solution containing 32 μg/mL of gliclazide and 200 μg/mL of metformin was prepared. Five replicate injections of the mixed standard solutions were injected and the parameters were evaluated. The system suitability test was done on three different days. The observed system suitability parameters are shown in Table 1.

Table 1. System suitability.

Day	Analyte	RSD (%)	Tailing factor	Theoretical plates	K'
		Combination I			
1	Glipizide metformin	0.40	1.22	2,094	5.0
		0.36	1.28	5,969	22.8
2	Glipizide metformin	1.01	1.33	1,775	4.8
		0.32	1.47	5,591	23.5
3	Glipizide metformin	1.20	1.27	1,730	4.8
		0.37	1.56	5,194	22.7
		Combination II			
1	Gliclazide metformin	0.33	1.13	3,180	12.4
		0.34	1.38	5,518	22.9
2	Gliclazide metformin	0.32	1.15	3,170	12.6
		0.32	1.47	5,591	23.5
3	Gliclazide metformin	0.28	1.14	3,108	12.8
		0.37	1.56	5,194	22.7

Analytical Data

The calibration curve of glipizide and metformin for the analysis of combination I was obtained by triplicate injections of standard solutions with varying concentrations of glipizide and metformin in the range of 0.5–3.0 μg/mL and 50–300 μg/mL, respectively. From the regression of the calibration curve, the correlation coefficients (*r*) for peak area, peak heights of glipizide and metformin were 0.9998, 0.9999 and 1.00, 0.9954, respectively.

The calibration curve of gliclazide and metformin for the analysis of combination II was obtained by triplicate injections of standard solutions with varying concentrations of gliclazide and metformin in the range of 8–48 μg/mL and 50–300 μg/mL, respectively. From the regression of the calibration curve, the correlation coefficients (*r*) for peak area, peak heights of gliclazide and metformin were 0.9999, 0.9999 and 0.9999, 0.9969, respectively.

Reproducibility

The reproducibility was evaluated from two series of six aliquots of combination I and II. The coefficient of variation was 1.30% for glipizide and 1.39% for metformin in combination I. The coefficient of variation was 1.75% for gliclazide and 1.28% for metformin in combination II.

Analysis of Tablets of Combination I and II

The procedure was applied to the determination of glipizide, metformin (combination I) and gliclazide, metformin (combination II) tablets obtained in the Indian market. Figure 5(A) shows the chromatogram of combination I (glipizide and metformin) and Fig. 5(B) shows the chromatogram of combination II (gliclazide and metformin). The procedure was applied for the tablets of combination I and II by different manufacturers. The results are shown in Table 2. The glipizide and metformin content was determined by taking six aliquots of each formulation and injecting them into the chromatographic system. The results were reproducible and the recoveries, with respect to the values declared by the manufacturers for glipizide and metformin (combination I), were in the range of 96.1–103.4%, 95.0–100.0% using peak areas and 96.4–103.6%, 97.3–102.5% using peak heights, respectively.

The recoveries for the gliclazide and metformin (combination II) were in the range 94.7–100.8%, 96.2–101.2% using peak areas and 93.1–101.5%, 92.2–102.5% using peak heights, respectively. The proposed procedure for the

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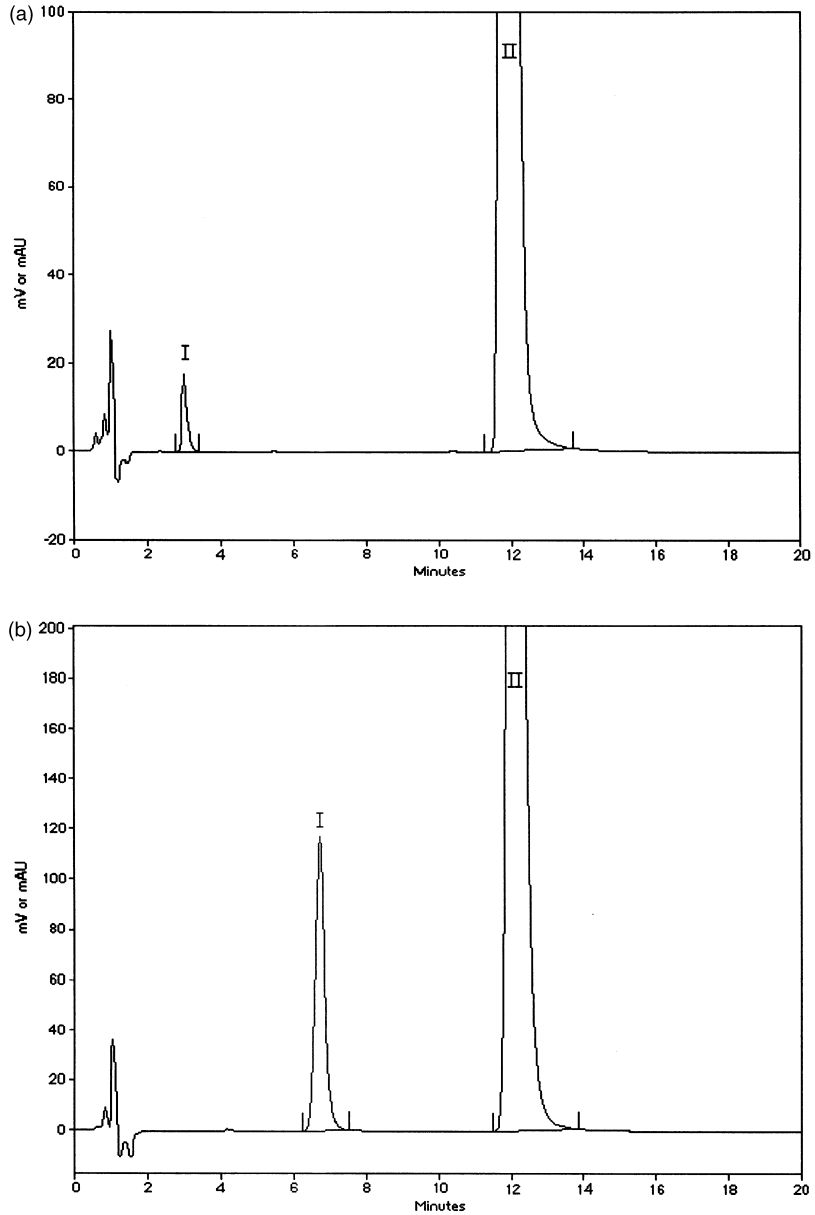

Figure 5. (a) Chromatogram of combination I showing the peaks of glipizide (I) and metformin (II); (b) chromatogram of combination II showing peaks of gliclazide (I) and metformin (II).

Table 2. Analysis of tablets of combination I and combination II.

Source	Label claim	Found (peak areas)	Found (peak heights)
	Combination I		
Product 1	Glipizide 5 mg	5.05 ± 0.07	5.08 ± 0.06
	metformin 500 mg	483.52 ± 8.41	495.54 ± 8.63
	excipients		
Product 2	Glipizide 5 mg	4.88 ± 0.06	4.90 ± 0.08
	metformin 500 mg	479.88 ± 6.15	491.00 ± 6.46
	excipients		
	Combination II		
Product 1	Gliclazide 80 mg	78.75 ± 1.31	78.55 ± 1.71
	metformin 500 mg	498.04 ± 5.34	502.07 ± 6.32
	excipients		
Product 2	Gliclazide 80 mg	78.45 ± 0.62	78.04 ± 1.05
	metformin 500 mg	499.09 ± 4.25	503.09 ± 4.23
	excipients		
Product 3	Gliclazide 80 mg	78.18 ± 1.80	77.80 ± 1.84
	metformin 500 mg	492.95 ± 9.69	497.76 ± 8.79
	excipients		

determination of metformin and glipizide in combination I and gliclazide and metformin in combination II is rapid and reliable.

CONCLUSION

The MLC technique described herein provides a simple, rapid, and reproducible determination of metformin in combination with glipizide and gliclazide dosage forms, which makes it potentially valuable in quality control of the drug dosage forms.

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