SPECTROPHOTOMETRIC QUANTITATION OF CHLORPROPAMIDE & PHENFORMIN

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ABSTRACT

A rapid, precise , accurate method developed and validated for estimating chlorpropamide and phenformin in combined dosage form. Full Spectrum Quantitation technique have been used. Wavelength range selected was220- 290 nm. The developed method is well suitable for routine analysis.

Full-Spectrum Quantitation (FSQ™) Multicomponent analysis system provides software for determining the concentrations of two or more components within a mixture using vector quantitation mathematics. The entire wavelength spectra of mixtures are used rather than a single wavelength for each component in order to yield better accuracy.

The user does not have to select an analytical wavelength(s). All calculations are done automatically including selection of the optimal number of vectors for the analysis in process.

KEY WORDS: Chlorpropamide, Phenformin, FSQ, MCA.

1.INTRODUCTION

Chlorpropamide and Phenformin are used in diabetic therapy. Chlorpropamide is a sulphonyl urea, which stimulates â- cells of Isletsand increases insulin secretion. Phenformin is a biguanide which increases glucose uptake and increases glycolysis and decreases gluconeogenesis. Both together have tremendous effect in diabetic therapy.

Indian pharmacopoeia suggests colorimetric estimation for chlorpropamide and phenformin hydrochloride tablets. British pharmacopoeia '93 suggests titrimetric method for chlorpropamide and British pharmacopoeia '80 Suggests non-aqueous titrimetric method for phenformin hydrochloride. USP '90 suggests chromatographic method for estimation of chlorpropamide.

Estimation of chlorpropamide and phenformin in combined dosage form by FSQ have not been reported. Therefore an attempt made here to explore utility of FSQ technique.

Full Spectrum Quantitation (FSQ) ?

FSQ is an algorithm using fourier transform of the sample spectra,principal component analysis (factor analysis) and B-matrix mathematics.FSQ uses mixtures of the components to generate the spectrum for calibration and subsequent analysis. FSQ performs a fourier transformation on the scanned data by transforming all absorption values to a 70 term modified fourier absorbance transform. B-matrix mathematics provides relation between the absorbance and concentration of components in calibration matrix.

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The steps involved in calibration with standards as like this;

1) Absorbance spectra of standard à Fourier transformed spectra of standard à Eigen vector standard.
2) Fourier transformed spectra of standard X Eigen vector standard à P.C score of standard.
3) Components concentration standard X (P.C score standard)$^{-1}$àRegression coefficient.

The steps involved in prediction of unknown as like this;

1) Absorbance spectra of unknown à Fourier transformed spectra of unknown
2) Fourier transformed spectra of unknown X Eigen vector standard à P.C score of unknown
3) P.C score of unknown X Regression coefficientàcomponent coefficients concentration.

To make the standard calibration matrix, a few number of mixtures of the components in different proportions are prepared and absorbance recorded. This absorbance spectra of mixtures are fourier transformed. By principal component analysis a set of orthogonal eigen vectors and eigen values are derived. Regression analysis correlates orthogonal representations to concentrations.

Calibration matrix also should validate with controls in which concentrations of the components in mixture should be different from that of concentrations used to make standard calibration matrix. Once the calibration matrix validated,then unknown samples can scan and absorbance transform calculated automatically. Thus orthogonal representations are calculated and analysed relative to standard calibration matrix. Concentration of unknown samples are read out.

Features of FSQ

- Full spectrum used for each standard, control and sample.
- No need to select an analytical wavelength for each unknown.
- Automatic optimization and selection of calculation parameters with user override.
- Analysis of two or more components in a mixture.
- Use of up to 32 standards for B-matrix calculation.
- User may rerun, add or delete standards with automatic recalculation of the matrix.
- Use of up to 75 controls in triplicate.
- Dilution correction available for individual samples.
- Use of up to three replicates per sample.
- Automatic calculation and flagging of each sample(s) C.V. if outside desired limit.
- Printout of standards data, statistical information, controls data, and sample data.

Method parameters, B-matrix and sample data can be stored in separate files in the instrument for easy recall and use.

Instrument

Beckman's DU 650 I spectrophotometer equipped with multicomponent analysis mode

Chemicals and Materials:

Sodium hydroxide (AR grade) obtained from Nice chemicals, Kerala. Chlorpropamide and Phenformin reference standards from Cadila Pharmaceuticals. Chlorformin tablets (cadila pharmaceuticals)having chlorpropamide 50 mg combined with 25 mg Phenformin.

2.EXPERIMENT

Each standard drug(chlorpropamide and phenformin) solution (10 ìg/ml) prepared using 0.02N sodium hydroxide .These analytes scanned in entire UV wavelength range and recorded ë-max. (ë-max spectras are shown in figure:1). From this wavelength range for subsequent analysis fixed as 220-295 nm. To make calibration matrix, ten mixtures of standard drugs in varied concentrations(concentrations used are shown in Figure:2 as actual concentrations) prepared and analysed. Three more mixtures with varied concentrations prepared and scanned to validate the standard calibration matrix(Also displayed in Figure:2) Overlaid spectra of mixtures used in calibration matrix are showed in Figure:3.

Methodology for analysis of sample:

Twenty tablets of chlorformin weighed and crushed into powder. An average weight equivalent to one tablet transformed into 100 ml standard flask and dissolved in 0.02 N NaOH . Sonicated for ten minutes,then volume made up and filtered. Two ml of clear filtrate diluted to 100 ml with 0.02 N NaOH. This analyte have been used for analysis. Thus amount present in the analyte displayed in the recorder. The amount of drug recovered calculated using the following formula:

$$\frac{\text{Observed amount X Average weight of tablet X Working standard potency}}{\text{Weight of the powder taken}}$$

3.RESULTS

The results of sample analysis is given below(mean of six experiments)

Name of constituents	Estimated amount	%claim	R.S.D
Chlorpropamide	50.6528 mg	101.31 %	0.2421
Phenformin	24.8855 mg	99.55 %	0.4492

To validate the developed method, various experiments(three times each experiment) have been performed in different standard parameters like Specificity,Accuracy, Consistency and linearity of method, Precision, Ruggedness, Stability of Analyte.

To confirm the specificity of method, a dummy tablet(without active ingredients i.e. chlorpropamide and phenformin) prepared and analyzed in the same manner as that of sample. There was no reasonable absorbance at all.

To confirm the accuracy of the method, a 100% formulation prepared by incorporating exact weight of excipients and active constituents. Then analysed in the same manner of sample analysis. Results shown in following table.

To confirm the consistency and linearity of the method, 80 %, 100% and 120% formulation prepared by incorporating 80% weight of excipients and active constituents in 80% formulation, 100% weight of excipients and active constituents in 100% formulation, 120% weight of excipients and active constituents in 120% formulation and analyzed as in the same manner of sample analysis.

To know the precision of the method six individual experiments have been done in the same manner as that of sample analysis.

To check the system precision six times readings have been taken for the same analyte.

To study the stability of analyte , readings have been taken at an interval of half an hour for two hours. Analyte was found to be stable upto two hours.

To study the ruggedness of method, samples have been prepared in solvents from different batches and did the sample analysis.

Inorder to confirm the sensitivity of the method, results of accuracy, consistency and linearity of the method have been considered. Sensitivity have been calculated using the formula $\Delta R / \Delta C$ (= 20).

Results of validation as follows:

Specificity	No reasonable absorbance		Method is specific
Accuracy of the method (mean of 3 experiments)			
Name of drug	% Recovery	R.S.D	Method is accurate
Chlorpropamide	99.7322%	0.2409	
Phenformin	100.1512%	0.1011	
Consistency & Linearity of method (mean of 3 experiments each in 80%,100% &120% formulation.			
Name of drug	%recovery in 80%	%recovery in 100%	%recovery in 120%	Method is consistent & linear.
Chlorpropamide	99.13142%	99.7322%		
Phenformin	102.5091%	100.1512%		
Precision of the method(mean of six experiments)			
Name of drug	% recovery	R.S.D	Method is precise
Chlorpropamide	101.3056%	0.2421	
Phenformin	99.5418%	0.4492	
Intrasystem Precision(mean of six readings)			
Name of drug	% recovery	R.S.D	Systen have good precision.
Chlorpropamide	100.06122%	0.1576	
Phenformin	100.8824%	0.2684	
Ruggedness of the method (mean of three experiments)			
Name of drug	% recovery	R.S.D	Method is rugged
Chlorpropamide	101.16%	0.7793	
Phenformin	99.59143%	0.4692	
Sensitivity of the method			
Δ R	Δ R / Δ C		Since Δ R / Δ C is very less.Therefore method is sensitive.
0.030525	0.0015262		
0.2788	0.013943		

From the above table it is very clear that developed method is accurate, precise, specific, rugged and sensitive.

4.CONCLUSION

The developed FSQ method is found to be an powerful tool in multicomponent analysis. It have certain merits over conventional methods like HPLC, derivative spectroscopy etc. Costly chemicals are not required as in the case of HPLC.Once the calibration matrix made, sample analysis can be done routinely without much time expenditure.

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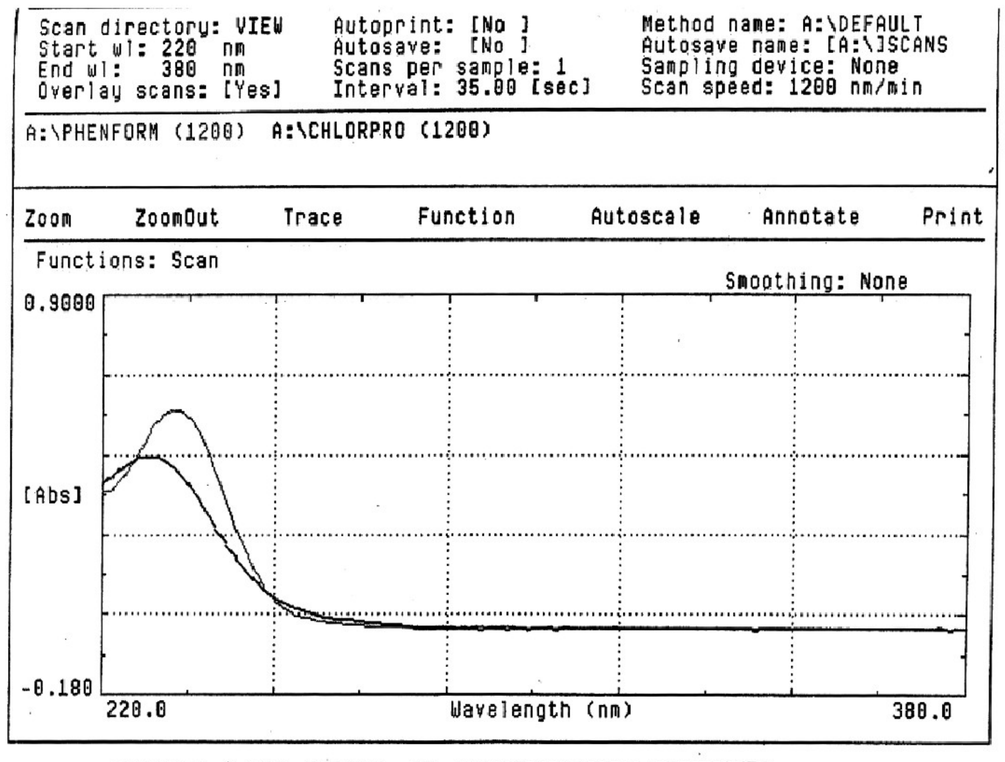


OVERLAID λ-MAX SPECTRA OF CHLORPROPAMIDE & PHENFORMIN

Calibration Matrix

STANDARD ERRORS OF ESTIMATE

Component:	CHLORPRO	PHENFORM
S.E.E.:	0.070	0.070

Total S.E.E.: 0.070 Average %Residual: 0.039

				CONCENTRATION (MCG/ML)			
Std#	Read	%Resid	Component	Actual	Calculated	Difference	Use
Read 1	YES	0.034	CHLORPRO	8.0000	8.0018	0.0018	[Y]
			PHENFORM	6.0000	5.9295	-0.0705	
Read 2	YES	0.049	CHLORPRO	9.0000	9.0024	0.0024	[Y]
			PHENFORM	0.0000	0.0590	0.0590	
Read 3	YES	0.037	CHLORPRO	9.0000	9.0432	0.0432	[Y]
			PHENFORM	5.0000	5.0454	0.0454	
Read 4	YES	0.036	CHLORPRO	9.0000	9.0958	0.0958	[Y]
			PHENFORM	6.0000	6.1103	0.1103	
Read 5	YES	0.034	CHLORPRO	10.0000	9.9641	-0.0359	[Y]
			PHENFORM	5.0000	5.0611	0.0611	
Read 6	YES	0.027	CHLORPRO	10.0000	9.8849	-0.1151	[Y]
			PHENFORM	6.0000	5.9851	-0.0149	
Read 7	YES	0.052	CHLORPRO	10.8000	10.8471	0.0471	[Y]
			PHENFORM	4.0000	3.9622	-0.0378	
Read 8	YES	0.037	CHLORPRO	10.8000	10.7475	-0.0525	[Y]
			PHENFORM	4.8000	4.7728	-0.0272	
Read 9	YES	0.050	CHLORPRO	12.0000	11.9696	-0.0304	[Y]
			PHENFORM	4.0000	3.9744	-0.0256	
Read 10	YES	0.029	CHLORPRO	12.0000	12.0510	0.0510	[Y]
			PHENFORM	4.8000	4.7308	-0.0692	

Standards file: A:\JLIN_STD Method name: A:\DEFAULT
Controls file: A:\JLIN_CTR

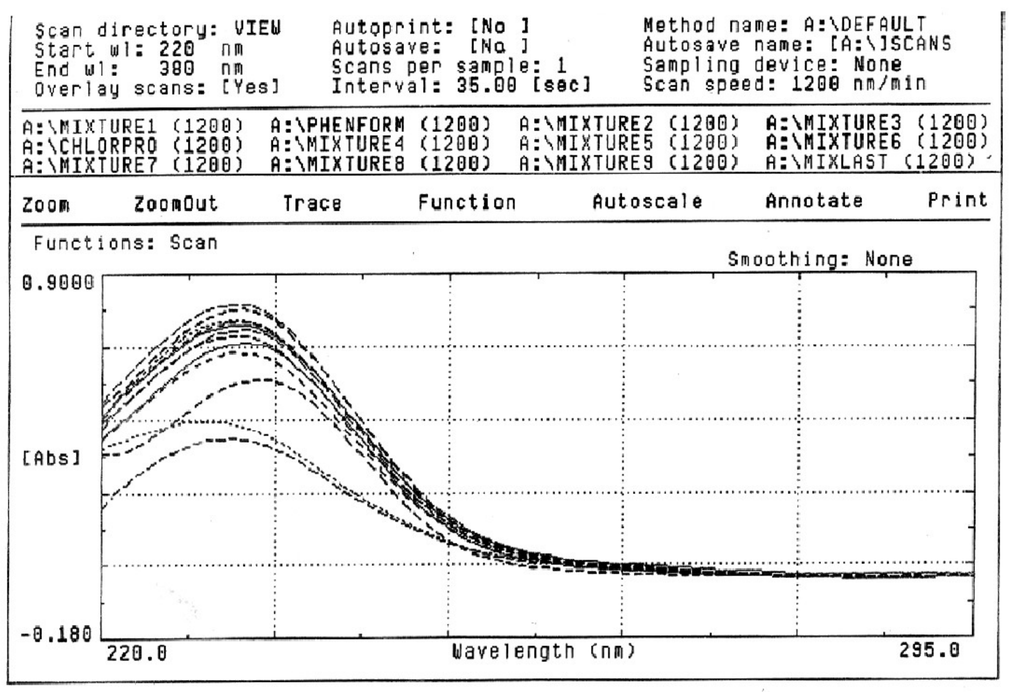
Starting wl: 220.0 nmm Number of control standards: 2
Ending wl: 295.0 nmm Units: MCG/ML
Sampling device: None Residual limit: 1.000 %

STANDARD ERRORS OF PREDICTION

Component:	CHLORPRO	PHENFORM
S.E.P.:	0.081	0.256

Total S.E.P.: 0.190 Average %Residual: 0.167

		CONCENTRATION (MCG/ML)				
Cntrl ID	Component	Actual	Calculated	Difference	%Resid	RF
1	CHLORPRO	8.0000	8.0808	0.0808	0.142	
	PHENFORM	5.4000	5.1296	-0.2704		
2	CHLORPRO	10.0000	10.0809	0.0809	0.192	
	PHENFORM	4.5000	4.2585	-0.2415		



OVERLAID SPECTRA OF MIXTURES IN STANDARD MATRIX & λ-MAX OF DRUGS

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