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A REVIEW ON ANALYTICAL METHODS FOR DETERMINATION OF DICYCLOMINE HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORMS AND BIOLOGICAL SAMPLES

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ABSTRACT

Dicyclomine Hydrochloride (DIC) is an antimuscarinic and antispasmodic agent. DIC is used to treat a certain type of intestinal problem called irritable bowel syndrome. It helps to reduce the symptoms of stomach and intestinal cramping. Its action is achieved via dual mechanisms; due to a specific anticholinergic effect at the acetylcholine-receptor sites and a direct effect upon smooth muscle. The drug is official in Indian Pharmacopoeia, United States Pharmacopoeia and British Pharmacopoeia. Many analytical methods have been reported for simultaneous estimation of DIC in its combined pharmaceutical dosage form but only fewer methods have been reported for estimation of DIC alone in pharmaceutical dosage forms. DIC is an important active pharmaceutical ingredient used even in children to relieve colic pain. This review therefore focuses on analytical methods reported for the estimation of DIC in literature and any further scope of developing novel instrumental analytical methods for estimation of DIC alone and in its fixed dose combinations.

KEYWORDS: DIC hydrochloride, Spectrophotometry, Chromatography, Potentiometry, Calorimetry, Antispasmodic.

INTRODUCTION

Dicyclomine Hydrochloride (DIC) is an antispasmodic and anticholinergic drug. It relieves smooth muscle spasm in gastrointestinal and urinary tract and is a smooth muscle relaxant. Chemically, DIC is [bicyclohexyl]-1-carboxylic acid, 2-(diethyl amino) ethyl ester

hydrochloride. DIC occurs as a fine, white, crystalline, practically odorless powder with a bitter taste. It is soluble in water, freely soluble in alcohol, chloroform, and very slightly soluble in ether. Its structural formula is shown in fig. 1.

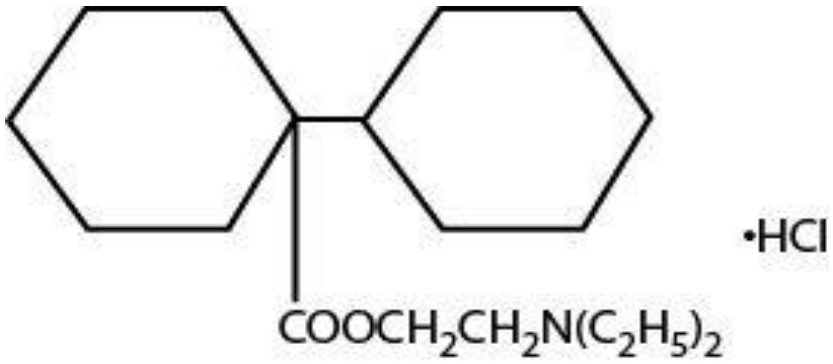

Fig. 1: Chemical structure of DIC

Its molecular formula is $C_{19}H_{35}NO_2$ and molecular weight is 309.4867 g/mol. Its melting point is 164-166°C. Animal studies indicate that smooth muscle relaxant action is achieved via a dual mechanism. It involves a specific anticholinergic effect (antimuscarinic) at the acetylcholine-receptor sites with approximately $1/8^{th}$ milligram potency of atropine (in vitro, guinea pig ileum) and also has a direct effect upon smooth muscle (musculotropic) as evidenced by DIC's antagonism of bradykinin- and histamine-induced spasms of the isolated guinea pig ileum. In cats and dogs DIC was found to be equally potent against acetylcholine (ACh)- or barium chloride ($BaCl_2$)-induced intestinal spasm while atropine was at least 200 times more potent against effects of ACh than $BaCl_2$. Tests for mydriatic effects in mice showed that DIC was approximately 1/500 times as potent as atropine; antisialagogue tests in rabbits showed DIC to be 1/300 times as potent as atropine.

Literature survey reveals that, in man, DIC is rapidly absorbed after oral administration, reaching peak values within 60 to 90 minutes. The principal route of elimination is via urine (79.5% of the dose). Excretion also occurs in the feces, but to a lesser extent (8.4%). Mean half-life of plasma elimination in one study was determined to be approximately 1.8 hours when plasma concentrations were measured for 9 hours after a single dose. In subsequent studies, plasma concentrations were followed for up to 24 hours after a single dose, showing a secondary phase of elimination with a somewhat longer half-life. Mean volume of distribution for a 20 mg oral dose is approximately 3.65 L/kg suggesting extensive distribution in tissues. DIC is used to treat intestinal hyper motility and symptoms of irritable bowel syndrome (also known as spastic colon). It has been used in combination with

Phenobarbital in the treatment of irritable bowel syndrome, acute enterocolitis and infant colic, but such combined therapy lacks substantial evidence of efficacy. DIC is in existence for quite some time now and is prescribed commonly for relieving spasm in infants, children and adults. Also it is available alone and in fixed dose combination with other drugs. Therefore this review describes analytical methods that have been reported till date for its quantitative estimation in pharmaceutical dosage forms and biological samples.[1, 2, 3]

METHODS FOR DETERMINATION OF DIC ALONE

Spectrophotometric method

Bebawy *et al* have reported a spectrophotometric method for DIC by using pi- acceptors. The method was based on the reaction of this drug as an n-electron donor with 2, 3-dichloro-5, 6-dicyano-p-benzoqunione (DDQ), p-chloranilic acid (p-CA), and chloranil (CL) as pi-acceptors to give highly colored complex species. Pi- donors are ligands filled with *p* or *d* orbitals and pi- acceptors are ligands having empty π antibonding orbitals of the proper symmetry and energy to interact with filled *d* orbitals of pi-donor ligands. The ratio was 1:1 (drug: acceptor) for DIC base with DDQ, *p*-CA, and CL reagents. The colored product formed is represented taking CL as an example in fig. 2. The interaction of DIC with chloranil (I) in dimethylsulfoxide yields blue dialkylaminovinylquinones (II), which has been used in a sensitive and specific photometric assay of this drug. The colored products were measured spectrophotometrically at 456, 530 and 650 nm for DDQ, p-CA, and CL, respectively. Optimizations of the different experimental conditions were studied. Beer's law was obeyed in concentration ranges of 20-100, 50-250, and 80-600 µg/mL for DDQ, p-CA and CL respectively.[4]

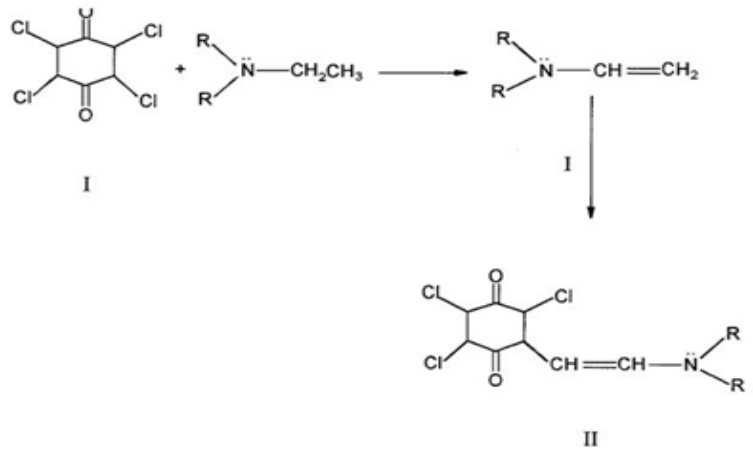

Fig. 2: Colored product of chloranil reagent with the amine[4]

Nuclear magnetic resonance spectroscopic method

Determination of DIC in tablet, capsule and injection dosage forms has been carried out by NMR spectroscopic method. The method consisted of an extraction step with chloroform, evaporation of the solvent, addition of maleic acid as an internal standard, dissolution of the mixture in deuterated chloroform-deuterated acetone (40:60), NMR spectral determination and integration of the peaks of interest. The concentration of DIC in the dosage form was calculated from the integral values for the peaks of the test compound and internal standard. The average recovery value ± standard deviation (n = 5) of DIC added to synthetic samples was 99.7 ± 0.9% (coefficient of variation 0.9%).[5]

Chromatographic methods

The reported chromatographic methods for determination of DIC alone are gas-liquid chromatography and HPTLC. Both the methods have been found to be sensitive, simple, selective, precise, and accurate. Gas liquid chromatography, an automated computerized method was developed for content uniformity determination of DIC in capsules and tablets. A 4 automatic sampler-equipped gas chromatograph interfaced with a minicomputer and 3% OV-17 column was employed for the determination and anthracene was used as an internal standard. Five sample injections were bracketed by standard mixtures containing about 90 and 110% of the labeled DIC. Data were taken on-line simultaneously from each gas chromatograph and a computer-generated report was produced. Calculations were done using a BASIC program with linear fit of the 90 and 110% of standard mixture. The GLC results were found to be comparable (within 1%) to those obtained using the procedure described in United States Pharmacopoeia.[6]

HPTLC analysis of DIC alone has been reported for injection formulation using precoated silica gel 60 F254 as stationary phase and toluene: acetone: methanol: conc. ammonia (5:2:1:0.02 v/v) as mobile phase. The dipping agent was sprayed on the plate for determination of DIC and plate was scanned at 523nm. The Rf value was found to be 0.71 for DIC. The linear detector response was observed between 800 ng $spot^{-1}$ to 4000 ng $spot^{-1}$ for DIC. The LOD and LOQ were found to be 250 ng $spot^{-1}$ and 800 ng $spot^{-1}$ for DIC. The recovery was carried out by standard addition method. The average recovery was found to be 99.45 % for DIC. Statistical analysis showed that the method was repeatable and selective for the quantitation of the drug in injection dosage form and for routine quality control of raw materials of the drug.[7]

Potentiometry

Potentiometric flow membrane analysis of DIC in serum, urine, milk and PVC membrane sensor for potentiometric determination of DIC in pharmaceutical formulation has been reported.[8,9] The first method consisting of five plastic membrane electrodes for the determination of DIC were fabricated and fully characterized in terms of composition, life span, usable pH range, working concentration range and temperature. The membranes of these electrodes consisted of dicyclominium-silicotungstate (Dc-ST), silicomolybdate (Dc-SM), phosphotungstate (Dc-PT), phosphomolybdate (Dc-PM) or tetraphenylborate (Dc-TPB) ion-associations dispersed in PVC matrix with dibutyl phthalate plasticizer. The electrodes showed near-Nernstian response over the concentration range of 4.0×10^{-6} to 1.0×10^{-2} M DIC. The method was applied to potentiometric determination of dicyclominium ion in pharmaceutical preparations, serum, urine and milk in batch and flow injection (FI) conditions with average recoveries of 96.1–102.7% and relative standard deviation of 0.055–1.994%. The electrodes exhibited good selectivity for DIC with respect to a large number of inorganic cations, organic cations, sugars and amino acids.

In second method, DIC-tetraphenyl borate ion-pair compound was first synthesized and then applied as a sensing element in preparation of PVC membrane sensor. The best PVC membrane sensor response was obtained by a membrane composition of 30% PVC, 63% DBP and 7% ion-pair. The detection limit of the sensor was obtained as 1×10^{-5} M. The proposed sensor had a fast response time of less than 15 s. The proposed method was successfully applied in determination of DIC in some pharmaceutical formulations.

Calorimetry

An alternative, reliable and stability-indicating calorimetric method have been developed based on selective extraction of DIC (free base) with cyclohexane followed by the formation of a yellow complex between the drug and bromocresol green.[10]

METHODS FOR DETERMINATION OF DIC IN COMBINATION WITH OTHER DRUGS

Spectrophotometric methods

Spectrophotometric methods have been reported for estimation of DIC in its combined dosage form.[11-16] Conditions for UV spectrophotometric analysis of DIC in various drug combinations are listed in table 1.

Chromatographic methods

Literature survey revealed that various RP-HPLC and HPTLC methods have been developed for simultaneous estimation of DIC with other drugs.[17-36] The methods have been found to be simple, accurate, robust and suitable for routine analysis of drug samples in their formulations. The conditions for RP-HPLC analyses of DIC in its combined dosage form are listed in table 2 and that for HPTLC analysis are listed in table 3.

Table 1: UV spectrophotometric methods for analysis of DIC in combined dosage forms

Combination	Method	λmax (nm)	Solvent	Linearity range (µg/ml)	Reference
Paracetamol (PARA) and DIC Tablet					
Method 1	Simultaneous equation	243 (PARA), 345 (DIC)	Methanol and 0.1 N NaOH	2-12 (PARA), 45-70 (DIC)	[11]
Method 2	Absorbance ratio	231.5 (isobestic point) 257 (PARA)	Methanol: 0.1 N NaOH (6:4 v/v)	2-12 (PARA), 10-35 (DIC)	[12]
Diclofenac sodium and DIC Tablet	Simultaneous equation	280 (Diclofenac Na), 267 (DIC)	-	5-25 (Diclofenac sodium), 2-10 (DIC)	[13]
Ciprofloxacin (CPX), Tinidazole (TNZ) and DIC Tablet	Absorbance corrected for interference (DIC), Absorbance ratio (CPX and TNZ)	274 (CPX), 340 (CPX and TNZ), 218 (DIC)	Methanol: 0.1 N NaOH (3:2 v/v)	2-10 (CPX), 2-20 (TNZ), 100-500 (DIC)	[14]
Nimesulide and DIC Tablet	Absorbance ratio, Simultaneous equation	300.8 (Nimesulide) 217.6 (DIC), 232.4 (isobestic point)	Methanol	10-100 (Nimesulide), 900-300 (DIC)	[15]
Mefenamic acid and DIC Tablet					
Method 1	Absorption correction, Differential derivative	223 (DIC), 308.60 (Mefenamic acid)	0.1 N NaOH, Methanol: NaOH (50:50 v/v)	1-6 (DIC), 25-150 (Mefenamic acid)	[16]
Method 2	Simultaneous equation	218.40 nm (DIC), 335 nm (Mefenamic acid)	NaOH	1-6 (DIC), 25-150 (Mefenamic acid)	[16]

Table 2: RP-HPLC methods for analysis of DIC in combined dosage forms

Combination	Detector type, Wavelength (nm)	Chromatographic column	Mobile phase	Reference
PARA and DIC tablet				
Method 1	UV-Visible, 218	C-18 Phenomenex (250 mm × 4.6 mm i.d., 5 µm)	Acetonitrile: Phosphate Buffer pH (5.5): triethylamine (75:25:0.02 v/v)	[17]
Method 2	PDA, 285	Novapack C18 column (250 mm × 4.6 mm, 4.0 µm)	Methanol: Water (24:76 v/v) pH 3.0	[18]
Diclofenac sodium and DIC	218	Hypersil BDS C18 (250 mm x 4.6 mm, 5.0 µm)	Acetonitrile 0.05 M : Potassium dihydrogen phosphate buffer (65:35 v/v) pH 7	[19]
Famotidine and DIC tablet	PDA, 270	Phenomenex Gemini column (250 mm × 4.6 mm, 5 µm)	Methanol : 0.1% triethylamine (46:60 v/v) pH 3.0	[20]
Omeprazole and DIC tablet	UV, 215	Phenomenex Luna C18 (250 mm × 4.6 mm, 5 µm)	Acetonitrile: 0.05M Phosphate buffer (45:55 v/v) pH 7.5	[21]
Diclofenac potassium and DIC				
Method 1	PDA, 263	Kromasil C18 (250 mm × 4.6 mm id, 5 µm)	Methanol: Water (70:30 v/v)	[22]
Method 2	254	Hypersil-ODC RP-C18 (250 mm × 4.6 mm, 5 µm)	KH_2PO_4 buffer: methanol: THF (50:45:05 v/v)	[23]
Mefenamic acid and DIC tablet				
Method 1	UV Visible and PDA, 215	C8 Luna (150 mm × 4.6 mm, 5 µm)	Acetonitrile: Monobasic Potassium Dihydrogen Phosphate (60:40 v/v)	[24]
Method 2	256	Lichrocart C18 (250 × 4.60, 5µm)	KH_2PO_4 : Acetonitrile (75:25 v/v)	[25]

Table 2: Continued……

Combination	Detector type, Wavelength (nm)	Chromatographic column	Mobile phase	Reference
Ranitidine hydrochloride and DIC tablet	UV, 218	Phenomenex C18 (150 × 4.6 mm, 5µm)	0.1% Ortho-Phosphoric acid: Acetonitrile (25:75 v/v) pH 3.5	[26]
Acetaminophen, Clidinium bromide and DIC tablet	UV visible, 218	Kromasil 100 C18 (250 mm × 4.6 mm, 5 µm)	Phosphate Buffer: Methanol: Acetonitrile (30:40:30 v/v) pH 7	[27]
Diclofenac and	UV-visible, 256	Lichrocart C18 (250	KH_2PO_4 Buffer:	[28]

Mefenamic acid Tablet	nm	mm × 4.6 mm, 5µm)	acetonitrile (75:25 v/v)	
Dextropropoxyphene HCL, PARA and DIC capsule	UV-visible, 231 nm	Phenomenex C18 (250 mm × 4.6 mm, 5µm)	NaH_2PO_4 Buffer: acetonitrile (60:40 v/v) pH 3.7	[29]
Clidinium bromide, Chlordiazepoxide and DIC tablet	PDA, 270 nm	Kromasil C18 (250 mm × 4.6 mm, 5µm)	Methanol: Acetonitrile: KH_2PO_4 Buffer (40:30:30 v/v/v) pH 4.0	[30]
Mefenamic acid, PARA and DIC	220	Brownlee C_{18} (250 mm × 4.6 mm, 5 µm)	Acetonitrile: KH_2PO_4 (70:30 v/v) pH 4	[31]

Table 3: HPTLC analysis of DIC in combined dosage forms

Sample matrix	Detection system & Wavelength (nm)	Stationary phase	Mobile phase	Reference
Omeprazole and DIC Tablet	Derivatizing agent: Potassium thiocyanate, Cobalt chloride and Sodium acetate (dissolved in water), Blue spots against light pink background were scanned at 345 nm within 20 min.	Silica gel 60 F254 plates, [20 cm × 10 cm with 250 µm thickness]	Toluene: acetone: methanol: ammonia (7: 1.5: 1: 0.1 v/v)	[32]
Diclofenac potassium and DIC Tablet				
Method 1	215 (Diclofenac potassium), 523 (DIC)	Precoated silica gel 60 F254 (0.2 mm thickness) on aluminium sheets	Toluene: acetone: methanol: conc. Ammonia (5.0: 2.0: 1.0: 0.02 v/v)	[33]
Method 2	410	Silica gel 60 F254 plates	Toluene: Methanol: Acetic acid (8:2:0.1 v/v)	[34]
Ranitidine hydrochloride and DIC Tablet	Iodine vapors and densitometric scanning at 410	Aluminum plates silica gel 60 F254	Methanol: Water: Acetic acid (8:2:0.1 v/v)	[35]
Nimesulide and DIC Tablet	Densitometric scanning Camag TLC scanner III at 345	Aluminum plates silica gel 60 F254	Toluene: Acetone: Methanol: Ammonia (7: 1.2: 1: 0.05 v/v)	[36]

Other methods

Promising four multivariate methods like CLS (Classical least square), ILS (Inverse least square), PCR (Principle component regression) and PLSR (Partial least square regression) were used for the determination of ternary mixture of Clidinium Bromide (CDB), DIC and Chlordiazepoxide (CDZ) in synthetic and marketed formulations. Overlapped data was

quantitatively resolved by using chemometric methods, viz CLS, ILS and PLSR method. Validation of the proposed method was successfully assessed for analysis of drugs in the various prepared synthetic mixtures and marketed formulation.[37]

CONCLUSION

A systematic review of analytical methods for determination of DIC in its formulations and biological samples is presented here. A wide range of instrumental methods for quantitative estimation of DIC have been developed successfully. But the methods reported are complex and perhaps time-consuming. The most suitable method for estimation of DIC alone in its dosage form is HPTLC. New trends and advances for quantification of DIC are based on HPTLC which are widely available and flexible. A vast number of HPLC methods have also been developed for analysis of DIC in its combination with other drugs. DIC is an important active pharmaceutical ingredient used in infants, children and adults to relieve colic pain. Much interest has therefore evinced in development of simple, rapid, precise and economical analytical methods for estimation of this drug. Though this drug is weakly absorbing in UV region, there is further scope in developing novel analytical methods for detection and assay of this drug alone in bulk and in its dosage form.

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