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Application of a Validated RP-HPLC Method in Solubility and Dissolution Testing for Simultaneous Estimation of Diacerein and Its Active Metabolite Rhein in Presence of Coformers in the Eutectic Tablet Formulation

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Abstract

The present study aimed to develop and validate a novel reversed-phase high-performance liquid chromatography method for simultaneous estimation of Diacerein (DIA) and Rhein (Rh, alkaline degradation product and active metabolite) in the presence of various coformers used to prepare eutectic oral formulation. Chromatographic separations were achieved on a Phenomenex Gemini C18 column (250 mm × 4.6 mm, 5 µm) placed in the thermostated column oven at 40°C. The mobile phase, comprising of acetonitrile and 10 mM ammonium acetate (pH 3.0), was eluted through the gradient system with 0.8 mL/min flow rate at 254 nm detection and analytical run time of 14 min. Additionally, the method was validated for specificity, linearity, precision, accuracy, selectivity, limit of quantitation, limit of detection and robustness as per International Conference on Harmonization guideline. The developed method was applied for the comparison of drug release profiles of pure DIA and from prepared eutectic formulations for the quantitation of DIA and Rh in the multicomponent adducts. The achieved method advocated their applicability in routine quality control analysis of DIA formulations without interference of degraded product and excipients.

Introduction

Drug molecules with limited aqueous solubility and dissolution are becoming common in the research and development portfolios of discovery focused on pharmaceutical industries. It is the inspiration for the pharmaceutical industries to generate a new solid form of drug molecules with optimized biopharmaceutical properties has essential significance in the advancement of orally administered drug products, the core of which is always a crystalline solid (1, 2).

In current scenario, designing of new solid form implementing the basis of crystal engineering approach has extensively been utilized in supramolecular chemistry as a new strategy to diversify the multicomponent solid forms that exist for a particular active pharmaceutical ingredient (API), and they can lead to improving the pharmaceutical attributes such as solubility, dissolution and bioavailability parameters without changing the efficacy of the drug (3, 4). The crystal engineering approach mainly involves the formation of novel polymorphs, solvate, hydrate, co-crystal, eutectic, solid solution and amorphous system. Among these, non-covalent derivatives mainly co-crystal and eutectic are most highlighted to improve the aforesaid properties of non-ionizable or poorly ionizable APIs with counter molecule/coformer using non-covalent interaction such as hydrogen bonding, π–π stacking, dipole interaction or Van der Waals forces (5).

Apart from the formulation and development of solid forms, an analytical methodology is a critical attribute for determining drug concentration in solubility, dissolution rate and formulation studies

(6). For pharmaceutical solid forms, require to determine the API solubility in the presence of different proportions of excipients. One of the challenging tasks in the dissolution study of novel solid form is a continuous change in the solution because of precipitation of the components during dissolution testing. The solubility and dissolution data generated can be erroneous due to the limitations of the analytical method. In this context, reversed-phase high-performance liquid chromatographic (RP-HPLC) method plays a vital role in the solubility and dissolution testing procedures. It imparts an extensive linearity range, advanced sensitivity and superior selectivity via separation. These features have been utilized to solve a variety of analytical problems faced during dissolution study of new solid forms (7). The method has been validated to ensure the applicability of intended use and give precise and accurate data for the dissolution study, which can be utilized to reflect bioavailability and bioequivalent study of the new pharmaceutical product (8).

Diacerein (DIA) and its active metabolite Rhein (Rh) are anthraquinone derivatives that have been used for the treatment of osteoarthritis. They activate the synthesis of proteoglycans and hyaluronic acid, cartilage components and inhibit synthesis of interleukin-1, cytokine involved in cartilage damage (9, 10). DIA is used to treat and prevent vascular diseases along with mild analgesic, antipyretic and anti-inflammatory activity. However, it suffers from the problem of poor solubility and dissolution, which limits its oral bioavailability (35–56%) (11). Crystalline powder of DIA (pKa of 3.01) shows low solubility under acidic condition and it degrades at alkaline pH in the intestine resulting in its conversion to Rh. The unabsorbed Rh irritates the mucosa and induces loose stools as a side effect. Longer residence time in the upper part of the gastrointestinal tract accompanied by gastric fluids results in increased absorption of DIA that leads to less amount of unabsorbed DIA to reach colon (12, 13). It seems to be a significant step to overcome the problems associated with DIA such as dissolution, bioavailability and laxative side effect. Several formulation approaches like solid dispersion, complexation, nanofiber, nanoemulsion, nanoparticles and many more have been attempted to improve the physicochemical and pharmacokinetic properties.

The present work was initiated to formulate novel eutectics of DIA with 2,4-dihydroxy benzoic acid (DHA) and fumaric acid (FMA) as different coformers using mechanochemical method, which has not been reported in literature up to now. Different characterization techniques were utilized to determine the eutectic solid form with addressing pH-dependent solubility and *in vitro* tablet dissolution profiles of the pure DIA. Several methods for determination of DIA alone and in combination with other drugs have been reported including spectrophotometric method (14, 15), chemometric-assisted spectrophotometry (16) and chromatographic method (17–20). A Literature survey revealed that no RP-HPLC method concerned with the quantification of DIA in the presence of its alkaline degradation product with different coformers was reported.

During solubility study, DIA was stable under acidic condition (pH > 5) but after that DIA was degraded to its alkaline degradation product Rh, which was considered the main product (12). Due to DIA instability and being Rh as the active metabolite, it is essential to quantify both in the prepared formulations. Also, the eutectics of DIA were comprised of various coformers and they required estimating without inferring the quantification of pure drug. As can be seen from the structural similarity between DIA and Rh, it will be a challenging task to determine both analytes in presence of other coformers by using simple spectrophotometric methods (21). So, the present work was aimed to develop RP-HPLC method and subsequently validated as a new, sensitive and economical analytical technique for simultaneous estimation of DIA and Rh in presence of coformers. The developed method was applied successfully for the comparison of solubility and dissolution studies of DIA and their prepared eutectic formulations.

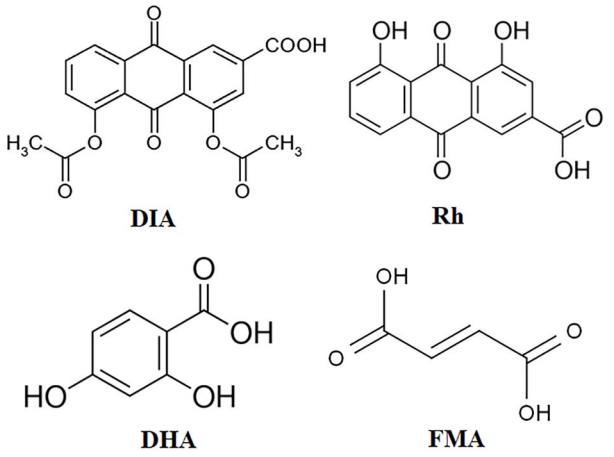

Figure 1. Chemical structures of diacerein (DIA); 2,4-Dihydroxybenzoic acid (DHA); fumaric acid (FMA) and Rhein (Rh).

Materials and methods

Materials

DIA (Pure drug) was generously provided by Ami Lifesciences Pvt Ltd (Baroda, India) with batch no. DSN/40400615. DHA and FMA as coformer were used as received from Sisco Research Laboratories Pvt Ltd (Mumbai, India). Rh (Active metabolite of DIA) was purchased from Yucca Enterprises (Wadala, Mumbai). The chemical structures of DIA, Rh, DHA and FMA were shown in Figure 1. Acetone and acetonitrile (HPLC grade) were obtained from Merck Pvt Ltd (Mumbai, India). All other chemicals and solvents used were of HPLC or analytical grade. HPLC grade water was generated from a Millipore Direct-Q ultrapure water system (Merck Millipore, MA, USA).

Preparation of multi-component solid forms

368.3 mg (1 mmoL) of DIA with DHA (462.4 mg, 3 mmoL) and FMA (232.14 mg, 2 mmoL) were ground separately for 30 min using agate mortar pestle by adding few drops of acetone to aid mixing between components. The obtained mass was scratched out and then dried in an oven (40°C) for 2 h followed by gentle trituration before sieving through a 100 mesh (ASTM standard) before further analysis. The resultant products were stored in the glass vials inside desiccators (23, 24).

Solid-state characterization

Thermal analysis of the co-crystallized samples was performed with help of differential scanning calorimetry (DSC 60, Shimadzu, Japan) previously calibrated for temperature and heat flow accurately using indium metal standard supplied by the instrument. Accurately weighed samples (2–3 mg) were placed in hermetically sealed aluminum pans and analyzed from 40 to 300°C at a scan speed of 5°C/min using a sealed aluminum empty crucible as reference. An inert temperature was maintained by purging nitrogen gas at a flow

rate of 100 mL/min. The powder X-ray Diffraction (PXRD) patterns of powder samples were collected on PANalytical diffractometer system (X'Pert pro Multi-Purpose Diffractometer, Philips) with Cu-Kα X-radiation at voltage 40 kV and current 30 mA. X'Pert HighScore Plus was used to collect and plot the diffraction patterns. The instrument was operated over a 2θ range of 10–40° at a scan rate of 4°/min. Fourier transmission infrared (FT-IR) spectrophotometer (Cary-630, Agilent) was used for recording the IR spectra of the samples. The ATR technique (Diamond ATR crystal, Agilent) was employed with Lab solution software. The spectrum was recorded in the region of 400–4,000 cm^{-1}.

Formulation and evaluation of in-house directly compressible tablets

Tablets of DIA and prepared eutectics were formulated separately using a direct compression technique. The formulation containing equivalent amount of DIA (50 mg) and different formulation excipients as follows: lactose (30–46%), microcrystalline cellulose-102 (2–10%), aerosol-200 (10%), sodium starch glycolate (10%), magnesium stearate (2%) and talc (2%). The specific amounts of sieved pure drug and prepared sample batches were mixed separately with sufficient portions of excipients to formulate 40 tablets from each batch. The individual blends were introduced manually into the die and compressed by 12 mm round and concave faced punch using eight-station rotary tablet machine (Karnawati engineering Ltd, India). Evaluation parameters of directly compressible tablets of pure DIA and prepared samples were performed and were shown in good accordance with acceptance criteria, see more details in supplementary data Table SI.

Instrumentation and chromatographic conditions

The HPLC system (Shimadzu Corporation, Japan) consisted of a LC-20 AD solvent delivery system, a DGU-20A5R vacuum degasser, a CTO-20 AC thermostated column compartment and SIL20AC autosampler coupled with a SPD-M20A PDA detector. The chromatographic data acquisition and processing were performed using Lab solution software (version 5.91) from Shimadzu Corporation, Japan. Separation was performed on a Phenomenex Gemini C18 column (250 mm × 4.6 mm, 5 µm) placed in column oven at 40°C using mobile phase comprising of acetonitrile (A) and 10 mM ammonium acetate adjusted pH 3.0 (B) with gradient elution as follows: 80% → 30% B at 0.0–8.0 min; 30% → 60% B at 8.0–12.0 min; 60% → 80% B at 12.0–14.0 min, see more details in supplementary data Table SII. Optimized flow rate and sample injection volume were set at 0.8 mL/min and 5 µL, respectively. Absorbance of analytes was detected at 254 nm and analytical run time was 14 min.

Preparation of standard solution

The standard stock solution of DIA and Rh were prepared by dissolving 50 mg separately in 2 mL DMSO and further diluted up to 10 mL with acetonitrile to achieve final concentration 5,000 µg/mL of each. The stock solution of FMA and DHA were prepared by dissolving 50 mg of FMA and 100 mg of DHA in solvent mixture (acetonitrile: water, 1:1% v/v) to give final concentration 5,000 µg/mL and 10,000 µg/mL, respectively. Further, working standard solutions were prepared in solvent mixture by serial dilution method to achieve concentration range 500, 250, 125, 50, 25, 10, 5, 2.5 µg/mL for DIA, 200, 100, 50, 25, 12.5, 8, 4, 2 µg/mL for Rh, 320, 160, 80, 32, 16, 6, 3, 1.6 µg/mL for FMA and 600, 300, 150, 60, 30, 12, 6, 3 µg/mL for DHA.

Method validation

The validation study consisted of system suitability, specificity, linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy and recovery, precision, solution stability as well as robustness as per International Conference on Harmonization (ICH) Q2 (R1) guideline (25).

pH-dependent solubility and dissolution study

The apparent solubility of DIA and prepared eutectics were measured in different buffer solutions in triplicate, namely pH 1.2 (Hydrochloric acidic buffer), pH 4.5 (Acetate buffer), pH 6.8 (Phosphate buffer) and Distilled water and buffers were prepared to utilize standard pharmacopoeial method as recommended by Indian Pharmacopeia 2010. An excess quantity of powder materials was dispersed to a screw-capped bottle containing 10 mL mentioned buffers separately. The resultant dispersion was kept in a shaker-incubator equipped with a temperature controlling system (Tempo Instruments and Equipments Pvt Ltd, India) at 37 ± 0.5°C with an agitation speed of 150 rpm for 24 h to allow saturation. The solutions were centrifuged at 14,000 rpm for 5 min (Centrifuge 5418R, Eppendorf AG, Germany) and supernatants were filtered through syringe filter (0.45 µm Millipore membrane filter). The filtrate was analyzed using RP-HPLC method for the quantification of drug in all samples.

The tablet dissolution profile of DIA and prepared eutectics were performed using dissolution apparatus USP type-II (USP Electrolab—TDT-06P, USA). The tablets were placed in dissolution vessels containing 900 mL of citrate buffer (pH 6.0) at 50 rpm maintained at 37 ± 0.5°C (22). 5 mL of aliquot was withdrawn from the suspension at specific time intervals up to 1 h and replaced with fresh above-mentioned dissolution media to maintain sink condition. The samples were filtered through syringe filter (0.45 µm) and quantification of drug was estimated using RP-HPLC method. The measurements were performed in triplicates.

Results

Solid-state characterization study of prepared eutectics

Among various characterization techniques, thermal technique (DSC) is the most helpful and approachable technique to interpret the formation of eutectic (26). DSC thermograph of DIA, DHA and FMA exhibited at a heating rate 5°C/min in the temperature ranges from 30 to 300°C, which depicts a sharp endothermic melting point at 255.17°C, 222.05°C and 289.48°C respectively. Acetone assisted ground product of DIA-DHA (1:3) and DIA-FMA (1:2) revealed a single endothermic event (201.53°C and 232.97°C, respectively), which is lower than both the participating components as presented in Figure 2A. These events suggested the formation of the new solid form either it could be cocrystal or eutectic. Further stoichiometric investigations using binary phase diagram (data not shown) showed a "V"-type pattern, which in turn confirmed the eutectic formation. It was further characterized by powder X-ray diffraction (XRD) that depicted all the characteristic peaks of the participating components that were present in diffraction pattern of prepared samples without any changes in the 2Θ values (Figure 2B). FT-IR spectra of prepared

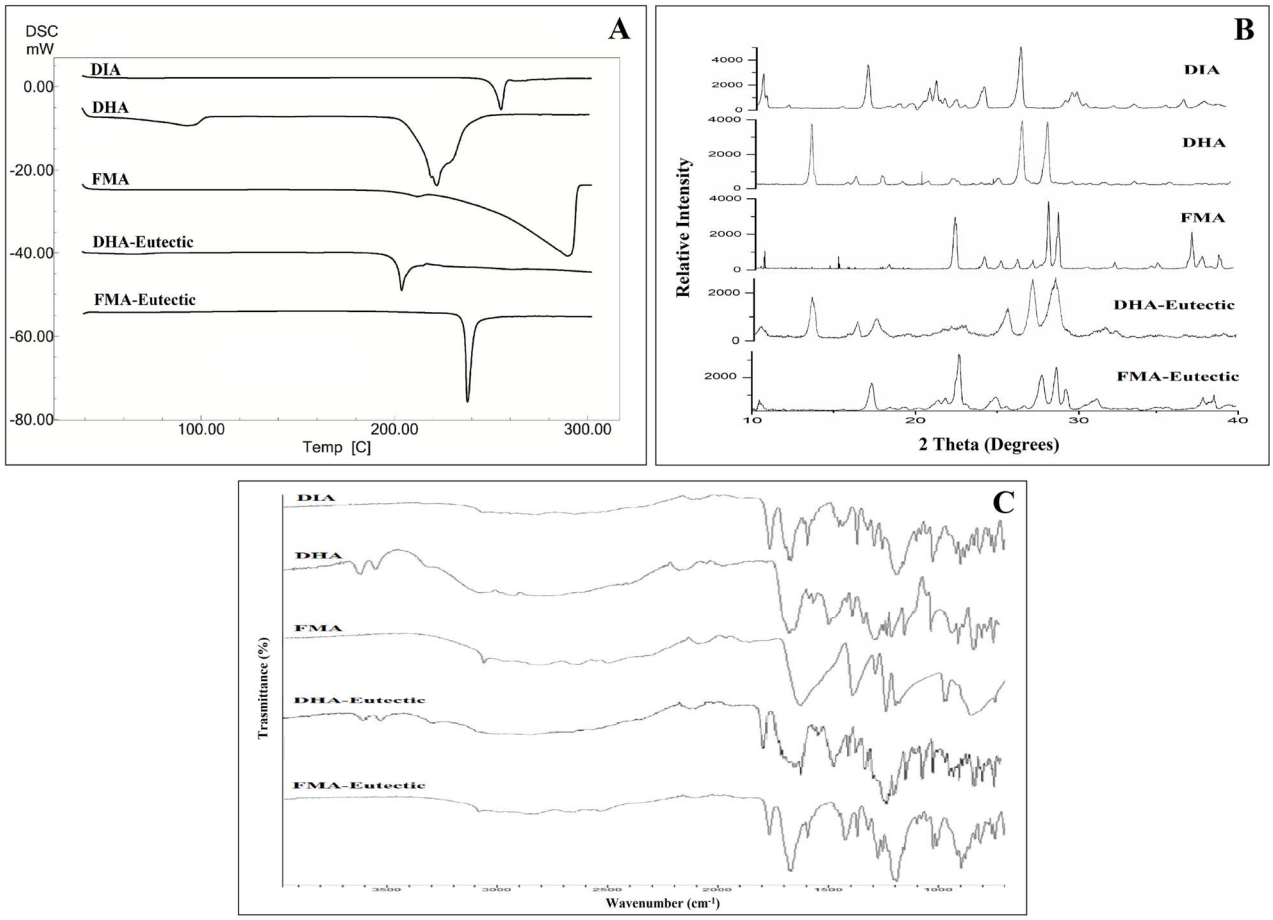

Figure 2. Solid-state characterization: DSC (A); powder XRD (B) and FT-IR (C) of DIA; DHA; FMA; DHA-Eutectic and FMA-Eutectic.

samples described the addition of characteristic vibration bands corresponds to DIA and coformer molecules (DHA and FMA) without any shifting (Figure 2C).

Development and optimization of chromatographic conditions

In the consideration of good sensitivity, better separation with acceptable peak shape, column efficiency and short analysis time, the final optimized chromatographic condition was attained with ammonium acetate (pH 3.0; 10 mM) and acetonitrile as a mobile phase, which was eluted by gradient on a Phenomenex Gemini C18 column (250 mm × 4.6 mm, 5 µm) placed in column oven at 40°C. To obtain a high specificity for each target analyte, UV detection was set at 254 nm with a short run time 14 min. The flow rate and injection volume were set at 0.8 mL/min and 5 µL, respectively. Figure 3 represents the HPLC chromatogram of a resolved mixture of DIA, DHA, FMA and Rh with the respective elution times at 10.5, 6.6, 4.1 and 11.6 min.

Method validation

System suitability

To establish the system suitability parameters of the proposed method, several statistical determination (number of theoretical plates, tailing factor and resolution) have been determined. These parameters are shown in Table I.

Specificity

The ability to separate all the substances (coformers, excipient, solvent mixture and mobile phase) from DIA in the sample was demonstrated by assessing the resolution between the peaks corresponding to various substances. The HPLC chromatograms recorded for the above-mentioned substances revealed that none of the peaks appeared at the retention time of DIA, Rh, DHA and FMA as illustrated in Figure 4.

Linearity and sensitivity

The calibration curves were constructed by plotting the concentration of the analytes versus the corresponding peak area responses. These sets of the eight standards were analyzed in triplicate and injected into the HPLC column. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. The sensitivity of the proposed method was estimated using LOD and LOQ determination. The LOD and LOQ were evaluated as the concentrations corresponding to signal-to-noise ratio of 3:1 and 10:1, respectively, and the results of all analytical parameters are summarized in Table II and software-generated linearity plot of all studied analytes is illustrated in Figure S1, see supplementary data.

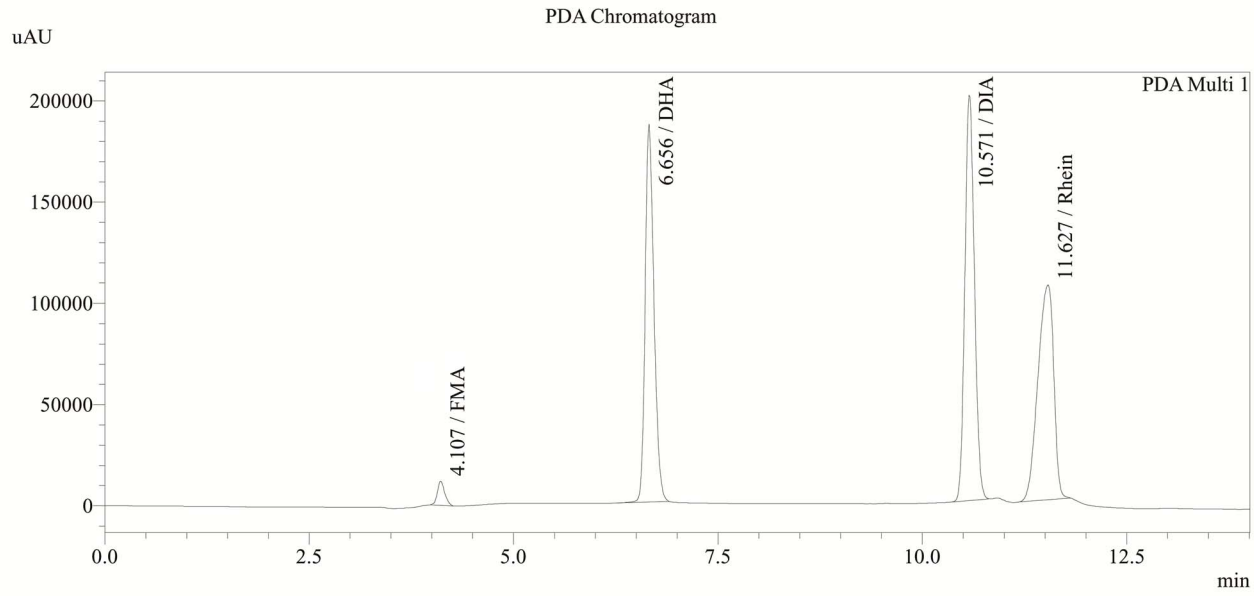

Figure 3. HPLC chromatogram of resolved mixture of FMA; DHA; DIA and Rh at retention times 4.1, 6.6, 10.5 and 11.6 min, respectively.

Table I. System suitability parameters for the studied analytes

Parameters	DIA	DHA	FMA	Rh	Limits
Theoretical plates	33,679	15,663	7,896	16,472	>2,000
Resolution (Rs)	16.46	13.27	12.83	4.94	>2
Tailing factor (T_f)	1.14	1.23	1.22	0.79	≤2
Retention time (min)	10.59	6.66	4.12	11.6	-

Good linearity was obtained with the aforementioned concentration ranges with a correlation coefficient (r^2) > 0.999.

Accuracy

Accuracy may investigate as %recovery by the assay of known, added amount of analyte. It is a measure of the exactness of the analytical method. The recovery experiments were carried out in triplicate by spiking three different concentrations of standard analytes solutions (within the linearity range) at 80%, 100% and 120%, respectively. The %RSD and overall percent recoveries of DIA with coformers and its metabolite were calculated as shown in Table III. The obtained results showed %recoveries ranging from 98.00 to 102.00 with %RSD < 1.8.

Precision

Six replicates at one concentration level were injected for intra-day and inter-day precision. The results of precision were expressed as percent recoveries of the particular components in the samples as expressed in Table IV. The %RSD values from intra-day and inter-day analysis were not exceeding 2% for all the studied analytes.

Solution stability

Solution stability was evaluated by monitoring the potency of both standard and sample solutions of the studied analytes over a period of one month. The solutions were stored at ambient temperature and tested at specific time intervals. The responses for the tested solutions were estimated using a freshly prepared standard. This finding demonstrates that sample and standard solutions retained a potency of 99.87 ± 2.0% as compared with the freshly prepared solution over a time of one month.

Robustness

The robustness of the proposed method was estimated by the analysis of studied analytes under a slight variation of chromatographic experimental conditions. The robustness of test method was demonstrated by carrying out; wavelength changes ±2 nm, column temperature variation ±5°C and flow rate variation ±0.2 mL/min and the obtained resulted are shown in Table V.

Method application

The validated HPLC method was successfully applied to determine pH-dependent solubility and dissolution profiles of pure drug and prepared eutectics in their formulations. The results are shown in Figure 5A for solubility and Figure 5B for dissolution experiments.

Discussion

As mentioned in our previous work (23, 24), novel eutectics of DIA with DHA and FMA using acetone assistant grinding method were developed and characterized using various characterization techniques to determine the eutectic solid form. DSC, PXRD and FT-IR data of the prepared samples anticipated that adhesive interactions between DIA and coformers (DHA and FMA) are not strong enough

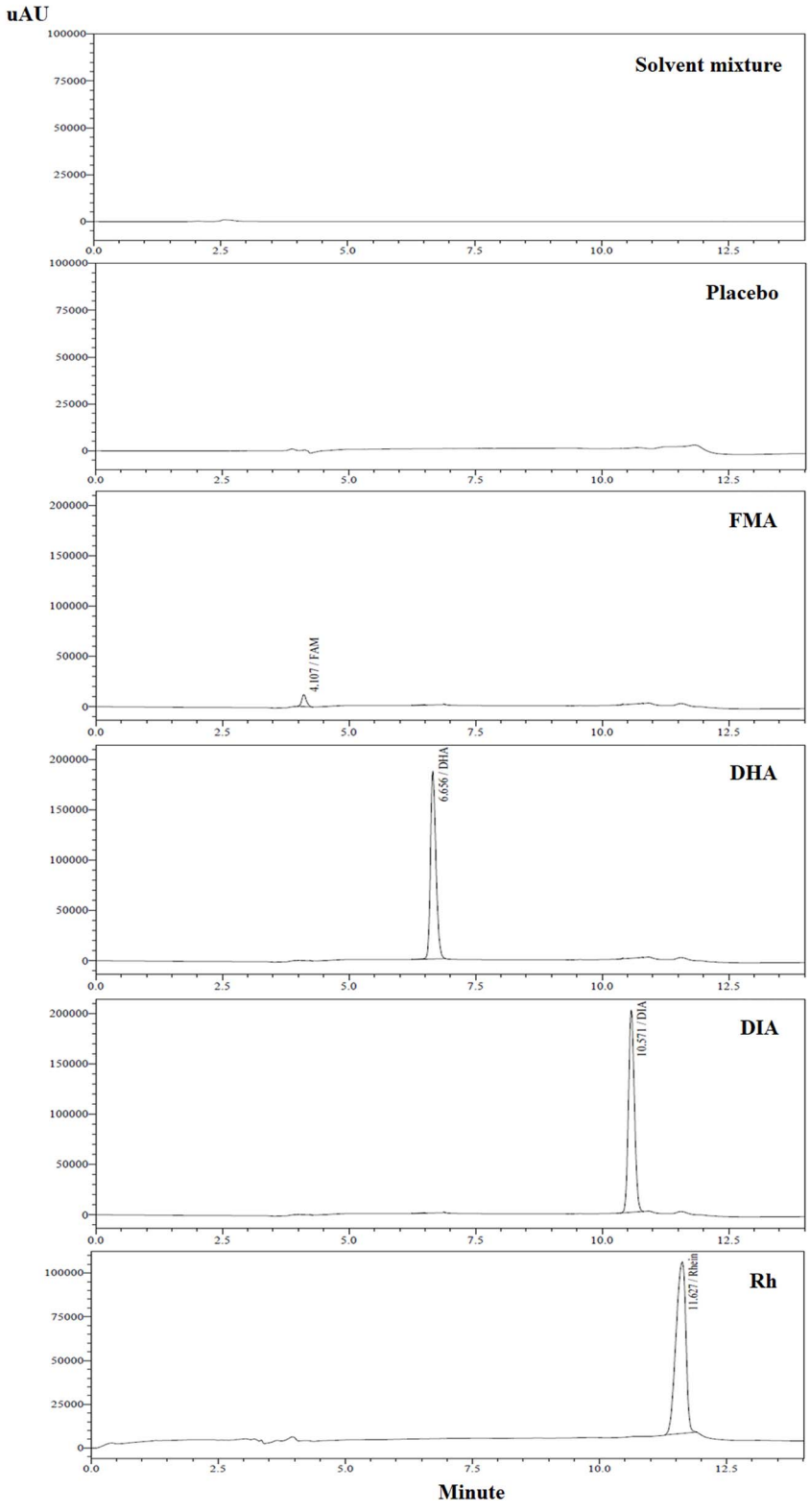

Figure 4. Specificity of the developed method taken under standard experimental conditions. Mobile phase: ammonium acetate (pH 3.0; 10 mM) and acetonitrile mixture, which was eluted by gradient elution on a Phenomenex Gemini C18 column at 40°C; UV detection wavelength: 254 nm; flow rate: 0.8 mL/min; run time: 14 min; injection volume: 5 µL.

Table II. Details of analytical parameters of the proposed method

Parameters	DIA	DHA	FMA	Rh
Regression equation[#]	$y = 1.075x$ — 3.834	$y = 0.971x$—0.648	$y = 1.012x$—0.469	$y = 0.980x + 0.034$
Correlation coefficient (r^2)	0.999	0.999	0.999	0.999
Linearity range (µg/mL)	2.5–500	3–600	1.6–320	2–200
LOD (µg/mL)	0.34	0.28	0.69	0.65
LOQ (µg/mL)	1.03	0.86	2.09	1.98

[#]Where *y* is the peak area and x is the concentration in µg/mL.

Table III. Accuracy evaluation data for the quantification of the studied analytes

Studied Analytes	Level (% w/w)	Taken Concentration (µg/mL)	Mean ± SD*	% RSD*
DIA	80	225	99.67 ± 1.12	0.78
	100	250	98.81 ± 1.23	1.12
	120	275	99.11 ± 0.98	1.43
DHA	80	270	101.03 ± 1.05	1.32
	100	300	99.18 ± 1.37	0.94
	120	330	98.75 ± 0.44	1.27
FMA	80	144	99.23 ± 1.59	1.37
	100	160	98.50 ± 1.23	1.70
	120	176	100.13 ± 0.97	1.56
Rh	80	90	99.65 ± 0.76	1.28
	100	100	100.20 ± 1.43	0.91
	120	110	99.37 ± 1.09	1.16

*Indicates data shown as mean ± SD and % RSD, ($n = 3$).

Table IV. Prsecision performance data for the quantification of the studied analytes

Studied analytes	Concentration (µg/mL)	Intra-day precision ($n = 6$) % RSD	Inter-day precision ($n = 6$) % RSD
DIA	125	0.81	1.50
DHA	150	1.07	0.87
FMA	80	1.24	1.77
Rh	50	1.80	1.04

Table V. Robustness data for the quantification of the studied analytes

Parameters	Variables	Analytes (% RSD) ($n = 3$)			
		DIA	DHA	FMA	Rh
Wavelength (nm)	252	1.34	0.68	0.88	1.06
	254	1.02	1.22	1.51	1.83
	256	0.53	0.61	0.82	1.24
Column temperature (°C)	35	1.46	1.05	0.96	1.14
	40	0.91	1.22	0.74	1.80
	45	1.72	1.37	1.55	1.42
Flow rate (mL/min)	0.6	1.64	1.41	1.03	1.70
	0.8	1.37	1.26	1.52	1.36
	1	1.84	0.99	1.28	1.32

to replace cohesive interactions and is ineffective in the transformation of the crystal packing of individual components and therefore resulted in eutectic binary mixtures (27, 28). Since, the main objective of this study is to emphasize the RP-HPLC method development and validation for the quantitative determination of drug in the prepared eutectic formulation, the characterization data of eutectics are not further discussed here.

Development and optimization of chromatographic conditions

Chromatographic conditions were optimized to attain good system suitability parameters such as resolution factor, peak tailing factor, retention time, height equivalent theoretical plates, sensitivity and peak shapes for all the analytes, as well as short chromatographic run time. The optimization parameters were significantly influenced

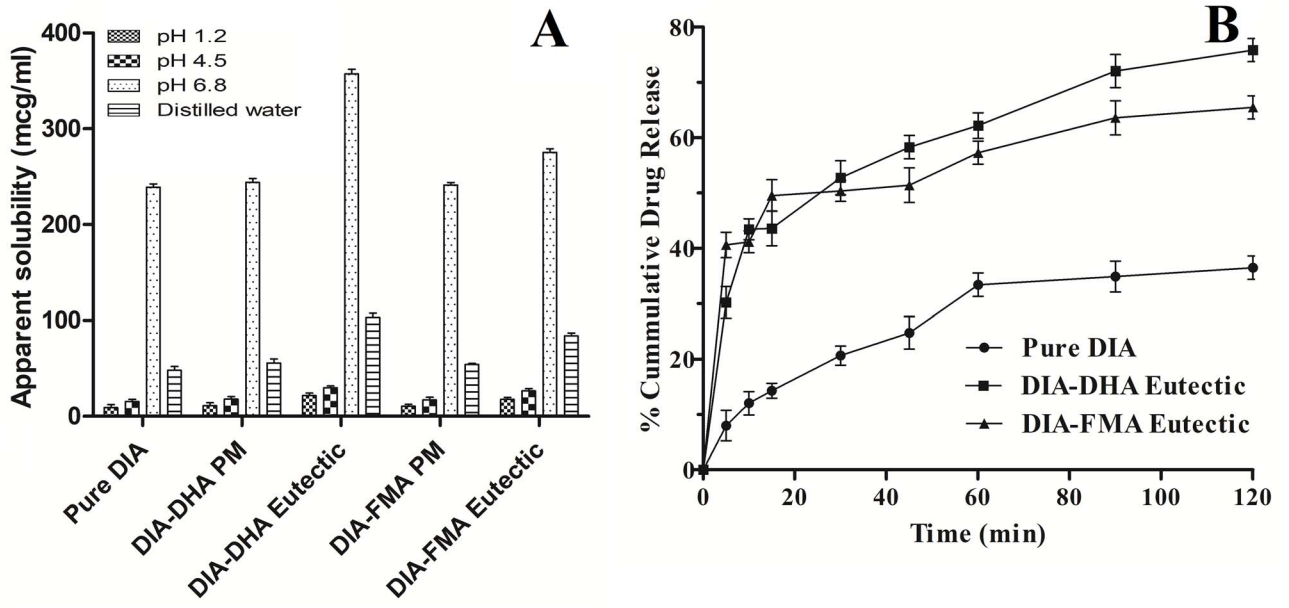

Figure 5. pH-dependent solubility (A) and dissolution (B) profiles of pure drug and prepared eutectics.

by the mobile phase composition (type and composition of organic modifiers/aqueous phase with pH of the solution, flow rate, column temperature and wavelength). Further, these parameters were altered to achieve the best system suitability parameters. Various trials were performed using different columns like Thermo, Atlantis, Gemini, Symmetry and Enable columns with several mobile phase combinations including methanol or acetonitrile as the organic phase. Regarding the aqueous phase of the mobile phase, trifluoroacetic acid, ammonium formate and ammonium acetate buffers at various pH values were tried. Acetonitrile and ammonium acetate buffer showed the optimum separation regarding resolution, retention times and peak shape of the studied analytes. By trying different pH ranges (2 to 6), pH 3.0 gave the symmetrical peak shapes for all analytes. Also, the resolutions between the analytes were not enough hence instead of binary elution, it was finalized to go with gradient system. The mobile phase, comprising of acetonitrile and ammonium acetate buffer (10 mM; pH 3.0) was eluted through the gradient system as follows: 80% → 30% B at 0.0–8.0 min; 30% → 60% B at 8.0–12.0 min; 60% → 80% B at 12.0–14.0 min. The flow rate and injection volume had little impact on the separation and were set at 0.8 mL/min and 5 µL, respectively. Good sensitivity was attained by selecting a common absorbance wavelength (254 nm) for all the analytes.

Method validation

A system suitability study was evaluated for the assurance of the high-quality performance of the chromatographic system. These parameters were found within the specific limits as shown in Table I. The obtained results suggested that the proposed method could be used for the quantification of studied analytes with a high degree of accuracy and precision. The specificity of the produced method was investigated by observing any interference encountered among excipients used for tablet preparation, solvent mixtures and mobile phase. The outcomes of the specificity anticipated that the proposed method was selective concerning the used coformers, excipients and solvents (Table II). The present RP-HPLC method bestowed a good range of linearity and sensitivity. The excellent accuracy in terms of high recoveries and precision (Tables III and IV) confirmed the suitability of the established method for the determination of DIA in their formulations. The solution stability study showed that for sample and standard solutions, the retention times and peak areas of the studied analytes remained almost unchanged and no significant degradation was observed within the given period, imparting that both solutions were stable for at least one month. The robustness suggested that the changes in the operational parameters did not lead to significant changes in the performance of the chromatographic system as the retention times and areas of each analyte were found to be preserved (Table V). It was concluded that the developed method was insensitive to slight changes in experimental conditions and considered to be robust.

Method application

The obtained results indicated that the drug release profiles were significantly enhanced in the case of prepared eutectics as compared to drug alone. The rise in solubility and dissolution of multi-component eutectic mainly depends on the presence of coformer, drug-coformer interaction and concentration of coformer (28–30). Restricted solubility of DIA in lower pH range, which ultimately leads to poor bioavailability, at the same time prepared eutectics showed enhancement in the solubility and dissolution for strong acidic to neutral condition. It has been considered that prepared eutectics might be a potential alternative to the commercial DIA. The estimated RP-HPLC method was employed successfully to compare the drug release profiles of pure drug and prepared eutectics in the presence of different coformers.

Conclusion

In conclusion, DIA-DHA and DIA-FMA eutectics were synthesized by mechano-chemical technique and characterized using DSC, PXRD and FT-IR. The prepared eutectics were evaluated through pH-dependent solubility and tablet dissolution studies. This work was tested the applicability and validity of a robust RP-HPLC method for the determination of DIA in the prepared formulations as per

ICH guideline. The proposed method exhibited a fast and applicable analytical technique for simultaneous estimation of DIA and Rh in the presence of various coformers. The developed method can obtain a good and reliable separation of each target analyte. The obtained method was simple, accurate and reproducible enough to compare solubility and dissolution profiles of DIA in the eutectic formulation. According to the results, the prepared eutectics of DIA showed improvement in the drug release profiles and considered a potential alternative to the commercial DIA. The achieved method advocated their applicability in routine quality control analysis of DIA formulations without interference from commonly encountered degraded product and formulation excipients in laboratories.

Supplementary Data

Supplementary Data are available at *Journal of Chromatographic Science* online.

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