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EMPAGLIFLOZIN: A REVIEW ON ANALYTICAL AND BIO-ANALYTICAL METHODS

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ABSTRACT: Diabetes mellitus is a metabolic disorder that causes high levels of blood glucose level in that 90% of the diabetic population accounts for type 2 diabetes mellitus it also has secondary complications like Cardiovascular disorder, Renal impairment, and susceptibility to infections. Empagliflozin is a potentially highly selective Sodium-glucose co-transportace-2 (SGLT-2) inhibitor used for the treatment of type 2 diabetes mellitus alone or in combination with the metformin or dipeptidyl peptidase-4 (DPP-4) inhibitors. The literature entitles the various analytical techniques like UV spectroscopy, High Performance Liquid Chromatography, High Performance Thin Layer Chromatography, Liquid Chromatography-Mass spectrometry. In this literature, we reviewed the various analytical, stability studies, impurity profiling and bio-analytical methods used for the estimation of Empagliflozin. This review gives the concise and collective information about the analytical validating parameters like Limit of detection (LOD), Limit of Quantification (LOQ), Standard Curve, Accuracy & Precision for the analysis of Empagliflozin alone or in combination with the Linagliptin or Metformin. This review helps to carryout further analytical studies on the mentioned drugs.

INTRODUCTION: Diabetes mellitus is a chronic metabolic disorder in which the ability of the pancreatic cells to produce or respond to the insulin hormone is decreased. According to WHO, 1.6 million deaths were directly caused and 2.2 million deaths caused due to contraindication with other diseases [1, 2]. In type 1, diabetes mellitus, the body's immune system destroys the pancreatic cells that produced insulin. Type 2 diabetes mellitus, which is also referred to as non-insulin-dependent diabetes, accounts for more than 90% of patients with diabetes [3, 4].

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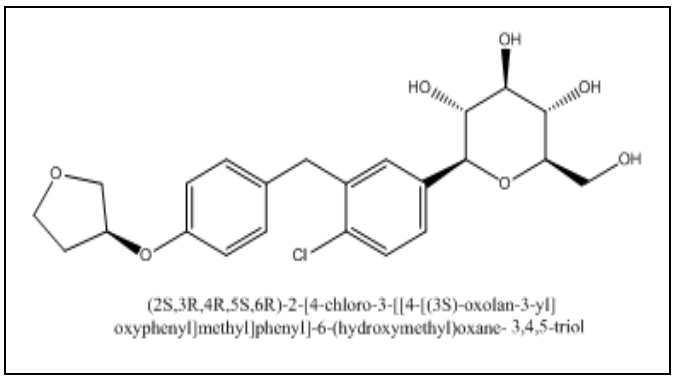
The patients with T2DM have more risk of secondary complications like cardiovascular risk and hypertension. The treatment guidelines for T2DM recommend Metformin as first-line therapy and sulfonylurea, SGLT-2 inhibitors, and DPP-4 agents as add on agents, and the SGLT-2 inhibitors also show lowering the cardiovascular risks in controlled trials [5-9].

Empagliflozin (EMP) **Fig. 1** is a Sodium-glucose co-transpoter2 (SGLT-2) inhibitor for Type-2 diabetes mellitus. It lowers the blood glucose level by reducing renal reabsorption from kidney it lead to increased urinary glucose excretion thus it reduces the plasma glucose level. Chemically it's (2S, 3R, 4R, 5S, 6R)-2-[4-chloro-3-[[4-[(3*S*)-oxolan-3-yl]oxyphenyl]methyl]phenyl]-6 (hydroxy methyl) oxane-3,4,5-triol. It's used alone or in combination with Linagliptin or Metformin in the treatment of type-2 diabetes mellitus [6-10].

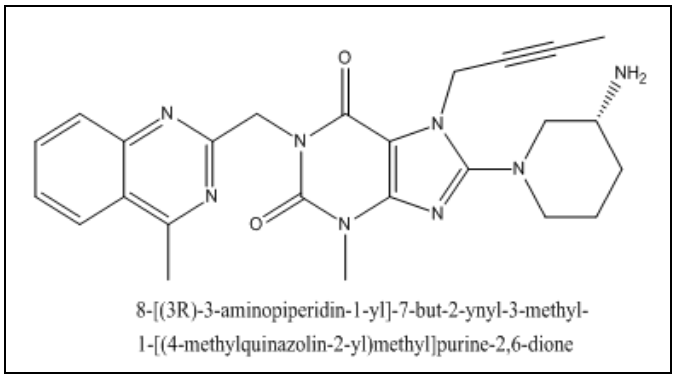
Linagliptin (LIN) **Fig. 2** reduces the blood glucose level by reversible inhibition of DPP-4 it decreases the degradation of the GLP-1 and GIP. This stimulates the beta cells in the pancreases and leads the breakdown of the glycogen in the liver [11, 12]. Metformin (MET) **Fig. 3** decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Unlike sulfonylurea's, Metformin does not produce hypoglycemia in either patient with type 2 diabetes or normal subjects and does not cause hyperinsulinemia [13-15].

Structures:



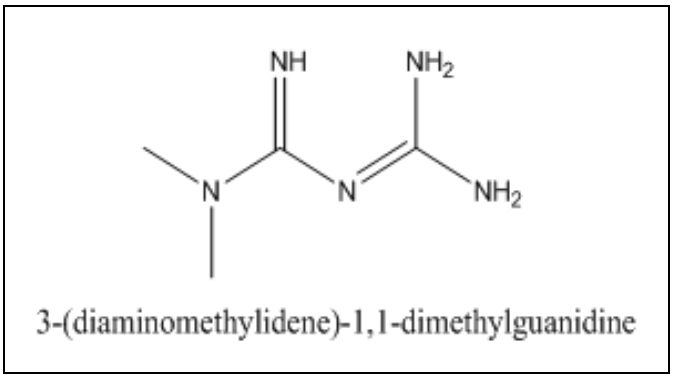
(2S,3R,4R,5S,6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3-yl] oxyphenyl]methyl]phenyl]-6-(hydroxymethyl)oxane- 3,4,5-triol

FIG. 1: EMPAGLIFLOZOIN



8-[(3R)-3-aminopiperidin-1-yl]-7-but-2-ynyl-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]purine-2,6-dione

FIG. 2: LINAGLIPTIN



3-(diaminomethylidene)-1,1-dimethylguanidine

FIG. 3: METFORMIN

METHODOLOGY:

UV Spectroscopic Methods: UV spectroscopy is a preliminary approach in the analysis of drug molecules; it's simple and effective in the analysis of molecules. It gives brief information about the solubility, lambda max of the entity, and UV absorbance pattern, so it's helpful in the identification and quantification of the drug substance. Only a few methods were reported for the analysis of EMP, and they are reviewed in this section.

Bossom M. Ayoub has developed two simple spectrometric methods for the determination of EMP and MET in their dosage form *i.e.*, SYNJARDY®. Constant multiplication coupled with spectrum subtraction technique is used, and it's found to be economical. LOD is found to be 0.12, 0.14 µg/ml for EMP, and MET [16]. Another method was developed by Bossom M. Ayoub for the analysis of EMP and MET in their co-formulation. Spiking technique is used for the enhancement of concentration, using the zero-order spectral scan estimation was carried out. Methanol was used as a blank for both drugs, and the method is validated according to ICH guidelines. LOQ and LOD values were found to be 0.51 µg/ml 1.52 µg/ml and 0.31 µg/ml 0.94 µg/ml for EMP and MET respectively [17]. A spectrophotometric and chemometric method has developed by B. M. Ayoub and method can be applied for the simultaneous estimation of EMP and MET in their tablet form by altering the zero-order spectra. The sample enrichment technique was used to increase the concentration. Linearity was observed between 2-12 µg/ml for both drugs.

LOD values were found to be 0.20 µg/ml and 0.19 µg/ml. LOQ was 0.59 µg/ml and 0.58 µg/ml for EMP and MET, respectively [18]. UV spectro-photometric method was developed by N. Padmaja and G. Veerabhadram, the method used for the determination of EMP & LIN in pharmaceutical dosage form by the simultaneous estimation method. Linearity was found to be 5-15 µg/ml, 2-6 µg/ml for EMP, and LIN, respectively, and % RSD below 2% indicate the validation of method [19].

TABLE 1: SPECTROPHOTOMETRIC METHODS

S. no.	Drugs	Method	Parameters	Author
1	EMP and MET	UV spectrum subtraction technique	Solvent: methanol Wavelength: 225, 237 nm Linearity: 2-12 µg/ml Correlation coefficient: 0.999	Bossom M. Ayoub [16]
2	EMP and MET	UV first-order spectral technique	Solvent: methanol Wavelength: 223.5, 233.5 nm Linearity: 2-12 µg/ml Correlation coefficient: 0.999	Bossom M. Ayoub [17]
3	EMP and MET	UV zero-order spectral method	Solvent: methanol Wavelength: 225, 237 nm Linearity:2-12 µg/ml Correlation coefficient:0.999	Bossom M. Ayoub [18]
4	EMP and LIN	UV simultaneous equation method	Solvent: methanol Wavelength: 233, 277 nm Linearity: 5-15 µg/ml, 2-6 µg/ml for EMP &LIN respectively Correlation coefficient:0.999	Padmaja and G Veerabhadram [19]

HPLC Methods: High-performance liquid chromatography is a simple and sensitive technique used for the quantitative estimation of chemicals, drugs, biological products. In HPLC, the separation is mainly achieved through partition and adsorption phenomenon. If the drug or any compound having more affinity towards the mobile phase will elute faster than the substances which have an affinity towards the stationary phase. There are two types of separation carried out based on the column type normal phase and reverse-phase in the normal phase the column is polar, and the mobile phase is non-polar, in the reverse phase, the column is non-polar, and the mobile phase is polar. The main advantages of the HPLC are high separation capacity and superior and sensitive reproducibility and less solvent consumption, and today, it's one of the major techniques used for the analysis of the drugs.

Analytical Methods: In analytical method development, all the reported methods carried out by the reverse phase technique for the LC method development and these methods help in the estimation of the drugs and selection of mobile phase for the estimation of the drugs and methods are validated according to the ICH Q2 R1 guidelines.

Susmita *et al*., developed stability indicating RP-HPLC method for the simultaneous estimation MET and EMP in the tablet dosage form. Standard BDS column 250 mm × 4.6 mm, 5µm particle size column and flow rate of 1 ml/min was used for the separation employing the mobile phase consists of 0.1% Orthophosphoric acid and Acetonitrile (50:50 v/v). Various stress conditions are evaluated. The retention time was found to be 2.58 and 3.6 min for MET and EMP respectively [20]. Another RP-HPLC method was developed by Syed Irfan Ali, Bharath Rathna Kumar P for the estimation of EMP and MET in their tablet dosage form using C18 column and 0.1% orthophosphoric acid and acetonitrile as mobile phase at flow rate of 1.1 ml/min and detection was carried out the 226 nm it's isobestic point of the two drugs. Linearity was found in the range of 125-750 ppm, 3.12-18.75 ppm for MET and EMP respectively. The method is validated according to ICH Q2 R1 guidelines [21].

RP-HPLC method was developed by S. K. Godasu and S. K. Sreenivas for the estimation of MET and EMP in their Bulk and dosage form using the C18 column and methanol and Phosphate buffer at pH3as mobile phase. The flow was adjusted to 1 ml/min. The retention time was found to be 2.403, and 3.907 min and percentage purity were found to

be 99.87% and 100.27% for MET and EMP, respectively [22]. Validated stability-indicating RP-HPLC method was developed by Shyamala *et al*., for the determination of EMP in its API form in this method BDS column is used as stationary phase and 0.1% orthophosphoric acid and acetonitrile as the (70:30 v/v) as mobile phase at the flow rate of 1 ml/min at the wavelength of 233 nm. Various degradation parameters like acid, alkaline, peroxide, photolytic, and neutral and oxidative degradation were carried out, and the drug is found to be more susceptible to Oxidative & Acidic conditions [23].

Anjali *et al*., has developed an RP-HPLC method for the determination of EMP and LIN in tablet dosage form using a C18 column (250mm × 4.6mm, 5µm). The mobile phase consists of a mixture of potassium dihydrogen phosphate buffer (pH 3.4) and methanol (70:30 v/v). Linearity was observed between 50-150 µg/ml [24]. Jaishwal *et al*., carried out stability, indicating HPLC method for estimation of process-related impurities in EMP drug substances. The chromatographic separation was achieved by using C8 column (250 mm × 4.6 mm, 5µm) column under gradient elucidation using 0.1% orthophosphoric acid and acetonitrile as the mobile phase and detection was carried out at 230 nm. The column temperature was maintained at 55 °C for better resolution, and all chromatographic parameters were validated according to the ICH guidelines [25].

Ismail Salama *et al*., carried out the simultaneous estimation of EMP Dapagliflozin Canagliflozin & MET. The separation was carried out using the C18 column (250mm × 4.6mm, 5µm) through isocratic elution acetonitrile and 0.05 M potassium dihydrogen phosphate buffer pH 4 (65:35 v/v) as mobile phase at the flow rate of 1 ml/min using UV detection at 212 nm. Linearity range was 7.5-225, 5-150, 6.5-187.5 and 10-1000 µg/ml for canagliflozin, dapagliflozin, and EMP & MET respectively [26]. An LC-UV method was developed by M. F. Abdel-Ghany *et al*., for the estimation of novel ant diabetic drugs in their combinations. Simultaneous estimation was carried out for the determination of EMP & LIN; subsequent simultaneous estimation was carried out for the determination of allogliptin and pioglitazone in their combination.

For the separation of LIN and EMP, the mobile phase consists of both acetonitrile and methanol with 0.1% formic acid at the pH of 3.6, at the flow rate 2 ml/min. in the second method for separation of allogliptin and pioglitazone simple mobile phase is used *i.e.*, methanol with orthophosphoric acid at pH 2.7, and the flow rate was maintained at 0.8 ml/min, and this two method can be applied for the estimation of the drugs in their dosage form [27]. P. Madhusudan *et al*., developed an RP-HPLC method for the simultaneous estimation of LIN and EMP in their tablet dosage form. C18 column was used for the estimation by employing the 0.1% orthophosphoric acid and acetonitrile (50:50 v/v) as a mobile phase in an isocratic elution mode. The retention time for LIN and EMP was found to be 2.2 min and 3.6 min, respectively. This method can be used for the analysis of Synjardy® tablets [28].

A UPLC method was developed by B.M. Ayoub for the simultaneous estimation of EMP, LIN, and MET in their pharmaceutical dosage forms using c18 column by applying isocratic elution using potassium dihydrogen phosphate buffer pH4 and methanol (50:50 v/v) as mobile phase. Linearity accuracy, precision was accepted over a range of 1-32, 0.5-16, 1-100 µg/ml for EMP, LIN, and MET. This method can be applied for the analysis of various dosage forms or combinations available [29].

Bio-Analytical Methods: There are only a few methods are reported in the bio-analysis of the drugs. Bio-analytical methods give the estimation or analysis of the samples from the blood, serum, plasma, urine, and other biological fluids; this helps in the clinical and preclinical studies of the drugs.

A simple UPLC method has developed by Fotouh R. Mansour *et al*., for the simultaneous determination of EMP and three related substances in Human plasma using dapagliflozin as internal standard tetrahydrofuran was used as a protein precipitating agent. The mobile phase consists of aqueous trifluoroacetic acid (0.1% pH 2.5), and acetonitrile (60:40) C18 column (50 mm × 2.1 mm 1.7 µm particle size), used for the separation at a flow rate of 0.5 ml/min and the method was found to be fast the run time is only 1.2 min, and it's also economical uses only 0.36 ml of acetonitrile per run. The method is validated according to the ICH guidelines [30].

RP-HPLC method was developed by Sharmila Donepudi, Suneetha Achanta, for the simultaneous estimation of LIN and EMP in human plasma by employing the c18 column and 0.1% orthophosphoric acid and acetonitrile (68:32 v/v) as the mobile phase. Detection was carried out at 218 nm using a 1ml/min flow rate. The retention time was observed at 4.12, 6.421 for EMP, and LIN, respectively [31].

TABLE 2: HPLC METHODS

S. no.	Drugs	Method	Chromatographic conditions	Author
1	MET and EMP	stability indicating RP-HPLC	Stationary phase: BDS column (250 mm × 4.6 mm, 5µm) Mobile phase: 0.1% Orthophosphoric acid and Acetonitrile (50:50 v/v) Wavelength:210nm Flow rate:1 ml/min Temp: Ambient	Susmita *et al*. [20]
2	MET and EMP	RP-HPLC	Stationary phase: C18 column Mobile phase: 0.1% Orthophosphoric acid and acetonitrile (45:55 v/v) Wavelength: 226 nm Flow rate:1.1 ml/min Temp: Ambient	Syed Irfan Ali, Bharath Rathna Kumar P [21]
3	MET and EMP	RP-HPLC	Stationary phase: c18 column Mobile phase methanol and Phosphate buffer (70:30 v/v) Wavelength:240 nm Flow rate: 1ml/min Temp: Ambient	S.K Godasu and S.K Sreenivas [22]
4	EMP	stability indicating RP-HPLC	Stationary phase: BDS column (250 mm×4.6 mm,5µm) Mobile phase: 0.1% Orthophosphoric acid and acetonitrile as the (70:30 v/v) Wavelength: 233nm Flow rate: 1 ml/min Temp: Ambient	Shyamala *et al*. [23]
5	EMP and LIN	RP-HPLC	Stationary phase: C18 column (250mm × 4.6mm, 5µm) Mobile phase: Potassium dihydrogen phosphate buffer (pH 3.4) and methanol (70:30 v/v) Wavelength: 240 nm Flow rate: 1 ml/min Temp: Ambient	Anjali *et al*.[24]
6	EMP	stability-indicating HPLC method for estimation of process-related impurities	Stationary phase: C8 column (250mm × 4.6 mm, 5µm) Mobile phase: 0.1% Orthophosphoric acid and acetonitrile (30:70 v/v) Wavelength: 230 nm Flow rate: 1.2 ml/min Temp: 55 °C	Jaishwal *et al*.[25]
7	EMP Dapagliflozin Canagliflozin & MET	simultaneous estimation	Stationary phase: C18 column (250mm × 4.6mm, 5µm) Mobile phase: Acetonitrile and 0.05 M potassium dihydrogen phosphate buffer pH 4 (65:35 v/v) Wavelength:212 nm Flow rate: 1 ml/min Temp: Ambient	Ismail salama *et al*.[26]
8	EMP & LIN	simultaneous estimation	Stationary phase: C18 column (250mm × 4.6mm, 5µm)	M. F. Abdel-Ghany *et al*. [27]

			Mobile phase: Acetonitrile and methanol with 0.1 % formic acid at the pH of 3.6, Wavelength:212 nm Flow rate: 2 ml/min Temp: Ambient	
9	EMP & LIN	RP-HPLC	Stationary phase: C18 column (250mm × 4.6mm, 5µm) Mobile phase: 0.1% Orthophosphoric acid and acetonitrile (50:50 v/v) Wavelength:212 nm Flow rate: 1 ml/min Temp: Ambient	P Madhusudan *et al*. [28]
10	EMP, LIN, and MET	UPLC	Stationary phase: C18 column (250mm × 4.6mm, 5µm) Mobile phase: potassium dihydrogen phosphate buffer pH4 and methanol (50:50 v/v) Wavelength:212 nm Flow rate: 1 ml/min Temp: Ambient	B.M. Ayoub [29]
11	EMP and three related substances	UPLC method in human plasma	Stationary phase C18 column (50 mm×2.1 mm 1.7 µm particle size), (60:40) Mobile phase: Aqueous trifluro acetic acid (0.1% pH 2.5) and acetonitrile (60:40) Wavelength: 210nm Flow rate: 0.5 ml/min Temp: Ambient	Fotouh R. Mansour *et al*. [30]
12	LIN and EMP	simultaneous estimation in human plasma	Stationary phase: C18 column (250mm × 4.6mm, 5µm) Mobile phase:0.1% Orthophosphoric acid and acetonitrile (68:32 v/v) Wavelength:218 nm Flow rate: 1 ml/min Temp: Ambient	Sharmila Donepudi, Suneetha Achanta [31]

LC-MS Methods: Liquid chromatography coupled with mass spectrometry is one of the advanced techniques used in drug analysis, and mass studies give information about the molecular weight and fragmentation patterns of a molecule.

Sensitive, rapid LC-MS method was developed for the simultaneous estimation of the empagliflozin and metformin in human plasma by taking Empagliflozin D4 and Metformin D6 as internal Standard. Using orosil C18 column and mobile phase consists of ammonium trifluoroacetate 10mM & Methanol (10:90 v/v) at the flow rate of 0.8ml/min. The sample preparation was done by using Solid-phase extraction technique by polymerised reverse-phase technique. The mass spectrometer was operated under positive ionization mode turbo ion spray ionization was used for the transition. For emagliflozin m/z 468.070/355.100 & m/z 130.072/71.200 for metformin, the method is validated successfully in the concentration range of 10.0.9-5013.46 ng/ml and validation is carried out under USFDA guidelines [32].

Pharmacokinetic evaluation of the EMP in Egyptian healthy volunteers using LC-MS/MS method the study conducted by Bassom M. Ayoub *et al.*, the study includes the main pharmacokinetic parameters like C_{max}, T_{max}, $t_{1/2}$, elimination rate constant, AUC_{0-t} and AUC_{0-inf}. the method was developed and validated using the dapagliflozin as the internal standard and liquid-liquidd extraction technique is used for the sample preparation. The m/z ratio was found to be 449.01 to 371.21 and 407.00 to 328.81 for EMP and dapagliflozin, respectively, using negative electron spray ionization mode [33].

Another LC-MS/MS method was developed by Bassom M. Ayoub and Shreen Mowaka using Bridge ethylene c18 column and aqueous formic acid and acetonitrile as the mobile phase in isocratic elution mode. The mass spectrometer was operated under the multiple reactions monitoring mode (MRM) using ESI technique, m/z 451.04–71.07 for EMP, and m/z 130.11–71.14 for MET in positive mode [34]. Mixed-mode solid-phase extraction LC-MS/MS method was developed for

the determination of EMP and LIN in human plasma by Pranav S. Srivasta *et al*., the method employs reverse phased solid-phase extraction technique using different sorbents. The chromatographic separation was carried out using cyano column, and the mobile phase consists of 2mM ammonium acetate buffer and acetonitrile. The mass spectrometer was operated under the positive ESI mode in MRM transitions m/z 451.3-71.1 for EMP and m/z 473.2- 420.2 for LIN. The standard curve was obtained between 1.50-1500 ng/ml.0.015-15.00 ng/ml for EMP and LIN, respectively [35].

HPTLC: HPTLC is a simple and advanced technique of TLC; this method uses very less amount of organic solvents compared to other methods. A stability-indicating HPTLC method was developed by R. P. Bhole for the analysis of EMP and LIN. The RF of LIN was 0.22 and 0.59 for EMP. The linearity was observed between0.2-1.2 µgper band and 0.1-0.6 µg per band for EMP and LIN, respectively. Stability indicating studies like acid, alkaline, photolytic, thermal degradation studies were carried out, and the method can be successfully applied for the analysis of EMP and LIN in their Co-formulation [36].

CONCLUSION: The above-mentioned data is concise collective information about the analysis of the empagliflozin alone or in combination with the linagliptin or metformin. All the methods mentioned are validated according to the ICH/USFDA guidelines and are useful in the analysis of the mentioned drugs. The prominent method for the analysis of the drugs is carried out by RP-HPLC in these methods the majorly used solvents are acetonitrile with 0.1% orthophosphoric acid and methanol with potassium dihydrogen phosphate buffer and in stability studies, the drug empagliflozin 224 nm sensitive in acidic and photolytic degradative conditions. In UV spectroscopic methods, methanol is the only solvent used; the lambda max for empagliflozin was found to be 233 nm.

There is only one stability-indicating HPTLC method in this method; they used the mobile phase methanol: ethyl acetate: glacial acetic acid (2:4:4 v/v/v). The empagliflozin was found to degrade in acidic and alkaline conditions. LC-MS methods with MRM techniques in the positive and negative mode under the electrospray ionization technique were reported for the analysis. In the bio-analysis, the extraction of the drugs is carried out through the Protein precipitation and solid-phase extraction, in the protein precipitation technique, acetonitrile is majorly used as the precipitating agent. In solid-phase extraction technique, MCX sorbent gives the best results compared to HLB and WCX sorbents.

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