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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ASCORBIC ACID

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ABSTRACT

The development and validation of analytical methods is an integral aspect of drug discovery and drug substance development in pharmaceuticals. Analytical technique creation and validation are two tasks that are intertwined in the research and development of new therapeutic products, as well as their combination and pharmaceutical quality control. Analytical technique is a procedure for determining drug content, contaminants, and degraded products, and validation establishes that the method is correct and may be used in quality control. As new medications emerge on a daily basis, the demand for analytical technique development grows, as conventional methods for these compounds are not available in pharmacopoeias. The development of a novel analytical method aids in the enhancement of analysis accuracy, precision, cost, and time consumption. This review article emphasises on literature findings of ascorbic acid method development and validation, which will aid in the development of new methods for determining ascorbic acid in bulk and medicinal dose forms.

KEYWORDS: Ascorbic Acid, method development, HPLC, UV, HPTLC, validation.

INTRODUCTION

Ascorbic acid is an antioxidant-rich reductone sugar acid. It has a colorless to light-yellow crystal or powder appearance and is water soluble. Ascorbic acid is one of the vitamin C forms (vitamer) and was the first chemical compound to be produced and identified as vitamin C in history. The term comes from the Greek words a- (meaning "no") and scorbutus (scurvy), which is an illness caused by a lack of vitamin C. When its structure was ultimately established by synthesis, it was credited to Haworth and Szent-Györgyi. Tyrosine oxidation

requires ascorbic acid as an enzyme cofactor. When combined with glucose and amino acids, it produces volatile molecules.

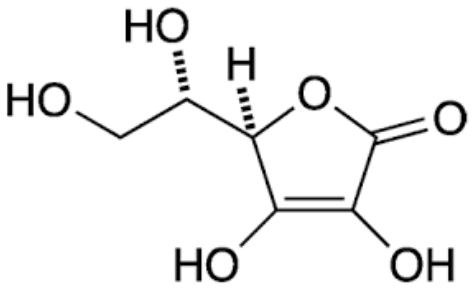

Figure 1: Structure of Ascorbic Acid.

Method Development of Ascorbic Acid

Zlatuse D.Clark (2016), develops a chromatographic method for measuring vitamin C levels in human plasma. Protein precipitation, internal standard addition, and dithiothreitol reduction were used to prepare the samples. Isocratic elution on a reverse-phase column was used to separate ascorbic acid, and coulometry was used to estimate concentration. Studies of assay linearity, sensitivity, imprecision, accuracy, analytical specificity, and carryover were used to verify the method. A single pump/single analytical column HPLC system was used to construct the new assay. The results were in good agreement with our earlier spectrophotometric approach. 1.0–2500 mol/L was the analytical measurement range. It took 13 minutes from injection to injection. Following that, a dual LC pump system with a 2-position/10-port switching valve capable of performing automatic alternating column regeneration was verified and implemented to boost method throughput and minimise turnaround time. The injection-to-injection time was cut in half, to just 6 minutes. The method was linear up to 5000 mol/L, with a 1.9 mol/L limit of quantitation. The total amount of imprecision was less than 5% .To precisely quantify vitamin C, the approach uses a streamlined sample preparation and a stable, non-endogenous internal standard. An automatic alternating column regeneration system was used to increase throughput.

Zbynek Gazdik (2008), For the detection of ascorbic acid, two electrochemical detectors (amperometric – Coulouchem III and coulometric – CoulArray) are combined with flow injection analysis. First and foremost, we improved the experimental circumstances. The optimal settings were 100 mV detector potential, 25 °C temperature, 0.09 percent TFA:ACN 3:97 (v/v) mobile phase, and 0.13 mLmin-1 flow rate. The tangents of the calibration curves for the coulometric and amperometric methods were 0.3788 and 0.0136, respectively. The

coulometric detector detected the calibration curve's tangent, which was nearly 30 times greater than the amperometric detector's tangent. As a result, we used a CoulArray electrochemical detector in conjunction with high-resolution liquid chromatography to estimate the detection limit for AA to be 90 nM (450 mol per 5L injection). The technique was employed.

Konda Ravi Kumar, Isocratic elution at a glide charge of 0.9ml/min turned into hired on a symmetry C 18 (250x4.6mm, 0.5µ in particle size) at ambient temperature. The cell section consisted of Water with acetic acid: methanol 95:5% (v/v). The UV detection wavelength turned into 245 nm and 20µl pattern turned into injected. The retention time for Ascorbic acid turned into 4.61+_ 0.22 min. The percent RSD for precision and accuracy of the technique turned into observed to be much less than the technique turned into proven as in line with the ICH guidelines. The technique turned into correctly implemented for habitual evaluation of Ascorbic acid in Health drinks.

Snezana (2010)Drugs and standards were eluted on Superspher RP18 (250 mm x 4.6 mm, particle size 10 µm) at 20 °C. Acetic acid (500 ml) was carefully added to 1.5 g of 1-hexanesulfonate sodium salt and stirred to prepare a mobile phase. Good (pH 2.6). The flow rate was 0.7 ml min 1. Emission was monitored using a UV detector calibrated to 280 nm. Each analysis took less than 4 minutes. The limit of quantitation was 1.95 µg ml 1. % recovery for various concentrations ranges from 99.58 to 101.93.Inexpensive and fast method and the high specificity for ascorbic acid in the presence of a variety of excipients make this method of HPLC is particularly suitable for the determination of ascorbic acid in test formulations and other similar formulations. Pharmaceutical/ veterinary preparations without prior sample preparation.

Robitaille (2016), Plasma vitamin C can be analyzed by electrochemical (EC) or high performance liquid chromatography (HPLC) with ultraviolet (UV) detection. We modified an existing UV-HPLC method for the analysis of plasma total vitamin C (sum of ascorbic acid and dehydroascorbic acid) to reconstitute a constant low pH sample followed by a simple procedure for isocratic reverse-phase HPLC separation using a pure aqueous solution. has developed. pH Mobile phase pH. ECHFLC is more widely recommended for plasma total vitamin C analysis than UVHPLC, but the 2 methods have not been directly compared. We formally compared the simplified UV-HPLC method and ECPLC in 80 serial clinical samples. The simplified UV-HPLC method has been shown to be less expensive, easier to set

up, require less reagents, require no pH adjustment, and provide better sample stability than many conventional methods for plasma vitamin C analysis. Eighty serial clinical samples representing a wide range of plasma vitamin C concentrations showed comparable results when compared to the gold standard ECHRPLC method.

KACHHAWAH (2016), aims to evaluate ascorbic acid (ASC) and folic acid (FLC) in cyanobacterial metabolites using a high-performance reversed-phase assay. Liquid chromatography (RPHPLC) methods, and their work has also been extended to nutraceutical formulations. The RPHPLC method was developed to simultaneously evaluate two vitamins ASC and FLC in cyanobacterial metabolites and nutraceuticals. Using isosbestic dots at a wavelength of 280 nm. This method was selected after system suitability calculations and validated according to ICH recommendations. The parameters of the developed analytical method are within the limits set by the ICH and USP standards. residence time was found. 2.334 and 3.892 for ASC and FLC respectively. The detection and quantitation limits for ISC and FLC are 0.087 and 0.263 µg/mL, 0.052 and 0.159 µg/ml, respectively. Recovery studies have shown that this method can extract analytes from the composition. Meeting method Verification criteria according to guidelines. This method is simple, accurate, specific and accurate. The newly developed method can be used in everyday pharmaceutical industry. ASC and FLC analysis of tablet dosage forms.

Laxman Sawant (2010), A reversed-phase high-performance liquid chromatography method has been developed and validated for simultaneous determination of ascorbic acid and gallic acid content in Phyllanthus emblica L. (Euphorbiaceae). A C18 column was used with a gradient elution with methanol as mobile phase and 0.1% (v/v) acetic acid in HPLC water at a flow rate of 0.9 ml min 1 . UV detection was performed at 278 nm. This method was tested for accuracy, precision, linearity, specificity, and sensitivity according to the guidelines of the International Conference on Harmonization. The amounts of ascorbic acid and gallic acid found in the freeze-dried extract of the plant were 4.58% and 0.59%. Total run time was 50 minutes. Ascorbic acid and gallic acid eluted with retention times of 3.60 and 10.77 minutes, respectively. Validation has shown that this method is specific, accurate, accurate, reliable and reproducible. The calibration plots were linear over the concentration ranges of 30–90 µg/mL for ascorbic acid and 5–15 µg/mL for gallic acid, respectively. The detection limits were 1.42 and 1.56 µg/ml, and the quantitation limits were 4.32 and 4.73 µg/ml for ascorbic acid and gallic acid, respectively. The recovery rates were 99.37% and 98.68% for ascorbic

acid and gallic acid, respectively, and the coefficient of dispersion was <2.0%. In negative ESI mode, the spectrum showed signals at m/z 174.98 for ascorbic acid and m/z 168.98 for gallic acid.

The high recovery rate and low coefficient of variation confirm the suitability of the method for the simultaneous analysis of ascorbic acid and gallic acid in Phyllanthus emblica.

Habtamu Abera (2020), Moringa stenopetala (Ms) is a drought-tolerant fast-developing indigenous tree in Ethiopia. The leaf a part of the plant attracted studies interest because of a couple of dietary and fitness blessings. The fitness blessings had been commonly attributed to the presence of compounds having antioxidant properties. One of such compounds is ascorbic acid. The intention of this observe became to expand a spectrophotometric approach to decide the extent of ascorbic acid withinside the suitable for eating leaf components of the plant. In this approach, a lower withinside the absorbance of Cr(VI) answer because of its response with AA became used as a foundation for the analysis. Mn(II) became used as a catalyst. The defined approach became proven in opposition to HPLC as a trendy technique. Factors influencing the discount of Cr(VI) with the aid of using AA, which includes incubation time, answer pH, and history awareness ratio, had been optimized. The theoretical detection restriction and restriction of quantification had been calculated to be 0.00154 and 0.0171 mg/ml, respectively. Out of the 3 one-of-a-kind regions of Ms leaf samples studied, sparkling Ms leaves from Arba Minch contained maximum awareness (237±0.001 mg/a hundred g) of AA, accompanied with the aid of using Konso (233±0.forty eight mg/a hundred g) and Dilla (21±0.forty eight mg/a hundred g), respectively. It became decreased substantially after boiling for 10 min and located to lower with growing cooking time. All Ms samples used on this observe contained a extraordinarily desirable variety or mild quantity of overall AA (200–250 mg/a hundred g). The effects acquired the usage of the modern-day approach had been in accurate settlement with that of HPLC methods (237 (UV–Vis) vs 239 (HPLC) and 233 (UV–Vis) vs 237 (HPLC)). The advanced approach is simple, fast, and may be correctly carried out for selective dedication of AA withinside the presence of different interfering species.

Mustapha (2016), created and optimised for determining the proportion of ascorbic acid in various brands of Vitamin C syrups. The chromatographic separation was done using HPLC water and methanol as the mobile phase, pumped at a flow rate of 1ml/min, and an ultraviolet detector for detection on an ODS (C18) Ultra spherical column, 5m (25cm x 2mm). The

calibration curve was linear over the range of 10-100g/ml, and the procedure was judged to be specific because no impurity or excipient interference peaks were seen. The devised approach was utilised to evaluate the quality of 55 samples of various varieties of Vitamin C syrup. 18.2 percent of the samples tested fell below the official B.P. requirement for ascorbic acid concentration, while 7.3 percent exceeded it. Fell inside the required limit, while 74.6 percent of the total samples examined were over it. For the analysis of ascorbic acid in Vitamin C syrups, we found this procedure to be easy, quick, and successful Mahmoud M. Sebaiy (2020) , For the determination of ascorbic acid, phenylephrine, paracetamol, and caffeine in their pure and tablet forms, an isocratic HPLC method has been developed. The separation was done on a Kinetex 2.6 C18 100A (4.6 mm 100 mm) column at room temperature with a mobile phase of 0.05 M potassium dihydrogen phosphate buffer (pH 3.50 by ortho-phosphoric acid): acetonitrile: methanol (70:20:10). For all medicines, the flow rate was 1 mL/min, maximum absorption was determined at 220 nm, and linearity was between 1 and 50 g/mL. Ascorbic acid, phenylephrine, paracetamol, and caffeine were shown to have retention periods of 1.83, 2.94, 3.74, and 5.13 minutes, respectively, indicating a very quick analysis time when compared to other methods. The detection limits were also reported to be 0.76. , 0.82, 0.47, and 0.24 g/mL, respectively, for ascorbic acid, phenylephrine, paracetamol, and caffeine, indicating a high degree of technique sensitivity. According to ICH criteria, the suggested technique was validated in terms of linearity, accuracy, precision, and robustness, and the findings were statistically compared to reference methods in terms of precision and accuracy.

Yogesh P Pancham (2020), Using the UV-1900 model, build and evaluate a spectrophotometric method for determining ascorbic acid in bulk powder form. The method was created using a solvent mixture of methanol and water (50:50 v/v), with ascorbic acid having a maximum absorption wavelength of 258nm. Validation of ascorbic acid was performed according to ICH recommendations parameters such as linearity, selectivity, specificity, precision, robustness, ruggedness, LOD & LOQ, and solution stability in order to optimise the established technique. Ascorbic acid is a kind of vitamin C. Precision, robustness, ruggedness, and solution stability were all found to be within acceptable limits in the validation report, which showed a linear response across concentration ranges of 3, 6, 9, 12, and 15g/mL with a r2 value of 0.997. A new spectrophotometric approach that was developed and verified was found to be selective, specific, linear, precise, robust, rugged, and stable. UV-1900 was used to determine ascorbic acid in bulk form.

Małgorzata Szultka (2013), The goal of this study was to use liquid chromatography combined with triple quadrupole mass spectrometry (LC-MS) to evaluate vitamin C stability with the goal of predicting breakdown products. In the multiple reaction mode, a UV detector (UV-Vis) was used in conjunction with a triple-quad mass spectrometer. MS-MRM transitions of m/z 175115 (quantifier) and 17589 (qualifier) for ascorbic acid were used in the negative ion mode of ESI. All of the validation parameters were within the Food and Drug Administration's acceptable range. In terms of linearity, LOD, LOQ, accuracy, and interday precision, the approach was fully validated. Within the prescribed concentration range, validation trials indicated good linearity with R2 = 0.999 and great repeatability (9.3 percent). The technique had a LOD of 0.1524 ng/mL and a LOQ of 0.4679 ng/mL. Within 6 minutes of analysis, LC-MS methodology appears to be an improved, easy, and rapid method for measuring the concentration of vitamin C and its breakdown products with good sensitivity, selectivity, and resolving power.

Tapan Seal (2016), For the simultaneous estimation of ascorbic acid, free phenolic acids, and flavonoids (catechin, rutin, quercetin, myrecetin, apigenin, and Kaempferol) in four different solvent extracts of two wild edible leaves, Sonchus arvensis and Oenanthe linearis, collected from the North-eastern region of India, a reversed-phase high-performance liquid chromatographic method using a photodiode array detector The chromatographic separation was done on a Dionex Ultimate 3000 liquid chromatograph with an Acclaim C 18 column (5 m particle size, 250 x 4.6 mm) and detection was done at three different wave lengths (272, 280, and 310 nm) using a mobile phase of acetonitrile and a 1 percent aqueous acetic acid solution with gradient elution. In a 1 percent aq. acetic acid extract of S. arvensis and O. Linearis, considerable amounts of ascorbic acid (1.2 percent and 2.3 percent, respectively) and gallic acid (0.02 percent and 0.06 percent, respectively) were found, as well as gallic acid (0.02 percent and 0.06 percent). The method's suitability for simultaneous quantification of ascorbic acid and all phenolic compounds in the two plants under investigation is confirmed by the high percentage of recovery (96-103%), low coefficient of variation (R2 > 0.99), and low limit of detection (LOD) and limit of quantitation (LOQ).

Khadija S. Raof, Ascorbic acid was determined using a high-performance liquid chromatographic technique. At room temperature, the analysis was carried out on a cosmosil 5C18-MS-II column (250 mm x 4.6 mm id, 5 m particle size). The best separation of ascorbic acid was achieved using the mobile phase acetonitrile (ACN), deionized water, and 0.01

percent KH2PO4 solution (80: 5: 15). (V: V: V). At room temperature, UV was detected at 253 nm with a flow rate of 0.75 ml.min-1. A concentration range of 1-500 g.mL-1 governs Beer's law. The detection limit and quantification limit, respectively, were calculated to be 0.0140 and 0.0468 g.mL-1. The method is precise (relative error percent = -0.02-2.58), recoveries are high (97.42-103.65), and it has been used to quantify ascorbic acid in pharmaceutical formulations, natural fruit, and vegetable.

JK. Mbinze For the quantification of vitamin C, a new and cost-effective UV/vis spectrophotometric technique using 0.1M hydrochloric acid as a dilution solvent has been developed. This effort developed pure and dose versions of the drug. The linearity test was carried out on a variety of variables concentrations ranging from 2 to 12 g/ml. The maximum amount of The wavelength of absorption discovered was 243 nm. Linearity, LOD (limit of detection) and LQ (limit of quantification) are two terms that are used interchangeably (LOQ), precision, accuracy, specificity / selectivity, and specificity / selectivity. Robustness was used as a validation criterion approach in accordance with the needs of the International Conference of Harmonization (ICH). To determine quantitatively, a newly discovered method was applied. Vitamin C is available in a variety of pharmacological formulations that are sold in the market. The findings of this study reveal that the approach is exact, precise, and repeatable (percent RSD 2%).simple, sensitive, durable, selective / particular, and fast.

MOHAMED H.S. AHMIDA The goal of this research is to find a good technique for determining ascorbic acid. For quick and easy ascorbic acid quantification, a sensitive HPLC-UV technique is devised (vitamin C) is found in a variety of vitamin C supplements. A Haisil is used in this procedure. For the stationary phase and the C18 column. The water is HPLC grade sulphuric acid: methanol (80:20) for the mobile phase to pH 2.2 The flow rate is 1.0 mL/min, with a 243 nm UV detecting wavelength. The upper limit of the detection level is expected to be 0.42 ppm. Precision values were displayed by the procedure (relative standard deviation, RSD percent) for effervescence pill was 1.79 percent.2.19 percent for a traditional tablet The outcomes are satisfactory in accord with the results obtained using the AOAC's certified titrimetry method the 1.67 percent accuracy (relative error, RE percent) and 1.41 percent for effervescence and traditional tablets, in that order.

Prawez Alam, For the simultaneous assessment of ascorbic acid and gallic acid in freeze-dried pomegranate fruit juice and herbal formulations, a new rapid, simple, sensitive, and high performance thin layer chromatography (HPTLC) has been established. The HPTLC

procedure was carried out on 20 x 10 cm glass covered silica gel 60 F254plates with ethyl acetate: acetone: water: formic acid, 10:6:2:2 (percent, v/v/v/v) and scanned at 254 nm for ascorbic acid and gallic acid. By comparing their single spots at Rf= 0.54 0.02 and Rf= 0.83 0.01, respectively, ascorbic acid and gallic acid in freeze-dried pomegranate fruit juice were identified. In the range of 100–800 ng/band, the regression equation (r20.9992) demonstrated a solid linear association between peakarea and the amount of ascorbic acid and gallic acid. Precision, accuracy, ruggedness, LOD, and LOQ were all evaluated. As a quality control tool, the suggested approach can be used to routinely determine ascorbic acid and gallic acid in various crude and herbal formulations. Ó2019

Chakraborthy G S, Medicinal plants are effective treatments for human ailments and play an important role in our daily lives. Amla is a popular household fruit belonging to the Phyllanthus emblica Linn family of Euphorbiaceae. Amla contains the anti-ascorbic agent Vitamin C, which is a powerful antioxidant. It is said to be the most abundant source of vitamin C. Thin layer chromatography (TLC) and a qualitative test were used to determine the amount of ascorbic acid in this fruit. As a result, it was quantified in different varieties of fruit collected from various geographical regions using the High Performance Thin Layer Chromatography (HPTLC) method. With silica gel 60 GF as the stationary phase, the procedure was carried out in TLC precoated aluminium plates. Ethanol: Acetic Acid (9.5:0.5 v/v) was used as the solvent systemwith an Rf of 0.760.03). The absorbance at 254 nm was used for quantitative analysis. In the concentration range of 0.5-5.0 g per spot, the linearity regression analysis for the calibration revealed r= 0.992 and 0.986 with respect to peak area and height. The larger variety taken from Shirpur has the highest concentration of ascorbic acid. The discovered approach can be used to analyse ascorbic acid in crude pharmaceuticals, as well as herbal and pharmaceutical dosage forms that incorporate amla as a component.

Min- Gul Kim, A method for determining ascorbic acid and its oxidation product, dehydroascorbic acid, in human plasma was developed using high-performance liquid chromatography (HPLC) with UV-vis detection. Human plasma ascorbic acid was extracted and stabilised with 10% metaphosphoric acid before being analysed on a Symmetry C18 column with a mobile phase of 5 mM Hexadecyltrimethylammonium bromide and 50 mM KH2PO4 solution (1.0 mL/min flow rate). The internal standard was isoascorbic acid, and the ultraviolet detector wavelengths were 254 nm and 265 nm. The difference in ascorbic acid concentration before and after dithiothreitol reagent reduction was used to compute

dehydroascorbic acid concentration. The concentration of ascorbic acid in human plasma was linear between 1 and 100 g/mL. The precision and accuracy of inter- and intra-day measurements were determined, and the conclusions were found to be within 15%. After oral delivery of 4,000 mg vitamin C tablets to healthy Korean volunteers, this approach was effectively applied to a human pharmacokinetic investigation of ascorbic acid and dehydroascorbic acid.

Alina Pyka-Pajak, For the simultaneous quantitative determination of acetylsalicylic acid and ascorbic acid in combination effervescent tablets, a new, simple, and cost-effective TLC-densitometric approach has been developed. The mobile phase was chloroform-ethanol-glacial acid at a volume ratio of 5:4:0.03 for separation on aluminium silica gel 60F254plates. For acetylsalicylic acid and ascorbic acid, UV densitometry was done in absorbance mode at 200 nm and 268 nm, respectively. Specificity, linearity, accuracy, precision, limit of detection, limit of quantification, and robustness were all used to verify the method according to ICH recommendations. Method validations show that both active compounds have good sensitivity, with low LOD and LOQ values. The linearity ranges for acetylsalicylic acid and ascorbic acid were determined to be 1.50–9.00g/spot and 1.50–13.50g/spot, respectively. The proposed method's satisfactory accuracy and precision are confirmed by a coefficient of variation of less than 3%. In relation to the label claim that acetylsalicylic acid and ascorbic acid meet pharmacopoeial standards, the findings of the combinedtablet formulation assay are 97.1 percent and 101.6 percent, respectively. The new TLC-densitometric approach might be used to analyse acetylsalicylic acid and ascorbic acid in mixed medicinal formulations on a regular basis. The proposed TLC-densitometry approach might be used as an alternative to modern high-performance liquid chromatography in the quality control of the above-mentioned compounds, and it could be used when HPLC or GC is not an option in the lab.

S Sridevi, For the simultaneous analysis of ascorbic acid (ASC), phenylephrine HCl (PHE), paracetamol (PAR), and levocetirizine HCl (LEV) in tablet dose form, a comprehensive and exact RP-HPLC technique was developed and validated according to ICH requirements. Using an Aegispak C18 column (25cm x 4.6mm, 5m) and phosphate buffer pH 4.00.05, Acetonitrile, a gradient technique was established. A wavelength of 220nm was used for detection. The load capacity was 50L and the flow rate was 1.5mL/minute. Ascorbic acid, phenylephrine HCl, paracetamol, and levocetirizine HCl were found to have retention times of 2.40.1, 5.40.1, 10.00.1, and 22.70.1, respectively. In concentration ranges of 50 to 150

percent, the technique was linear, and R2 was found to be within the limit. The accuracy level was determined to be between 98 and 102 percent. Because the percent RSD was discovered to be NMT 2.0 percent, the approach was precise. The placebo and diluent effects have no effect on the standard peaks, according to specificity experiments. In terms of linearity, range, precision, accuracy, and robustness, the created exact method was validated.

MG Gioia, A reversed-phase ion pair liquid chromatographic technique (RP-LC) is presented and validated for the measurement of dehydroascorbic acid (DHA) and ascorbic acid (AA), as well as acetaminophen, which is mixed in pharmaceuticals. AA and acetaminophen were determined without pre-column derivatization with 4,5-dimethyl-1,2-phenylenediamine, whereas DHA was determined after pre-column derivatization with 4,5-dimethyl-1,2-phenylenediamine (DMPD). The derivatization procedure was carried out in the dark in a sodium acetate buffer (80 mM; pH 3.7) solution containing EDTA as a metal scavenger under moderate circumstances (10 minutes at room temperature). The chromatographic separations were carried out on a Phenomenex Synergi 4u hydro-RP (150 mm 4.6 mm) utilising cetyltrimethylammonium bromide (CTAB) as an ion-pairing reagent in the mobile phase under isocratic elution conditions. For each chemical, linear responses were found. There was no significant difference between intra- and inter-day data, and the intra-day precision (R.S.D.) was 1.40 percent. All drugs performed well in recovery experiments (99.7%–101.8%), with R.S.D. ranging from 0.56 to 1.82 percent. For acetaminophen, AA, and DHA, the quantitation limits were roughly 40, 50, and 140 pmol, respectively. DHA impurity levels in dosage formulations were less than 0.2 percent of AA.

Fabiano O Silva, A novel gas chromatography technique for determining ascorbic acid was developed and compared to a high-performance liquid chromatography (HPLC) approach. After using basic clean-up procedures and a freeze-drying phase, the analysis was completed. Total ascorbic acid was measured following dithiothreitol reduction of dehydroascorbic acid, as in various HPLC procedures. Only ascorbic acid or multi-compound assays can be performed under these circumstances.

R Săndulescu, Using a carbon paste electrode (CPE-graphite: solid paraffin 2:1) as the working electrode and an Ag/AgCl reference electrode, electroanalytical studies of ascorbic acid, acetaminophen, and many combinations of these chemicals in various ratios were conducted. By testing samples between 10 and 50 l, potential curves were produced using various doses of ascorbic acid and acetaminophen. The oxidation processes were examined at

varied sweep speeds, at varying current sensitivities, under stationary working circumstances, and with stirring before each replication in a potential range of 0.1 to +1.3 V. The current has a linear variation for the following concentration ranges: +0.310.02 V for ascorbic acid and +0.600.05 V for acetaminophen; however, the current has a linear variation for the following concentration ranges: Ascorbic acid has a molecular weight of 103–102 M, whereas acetaminophen has a molecular weight of 3106–7.5103 M (r2=0.999 for both ascorbic acid and acetaminophen). Ascorbic acid and acetaminophen were mixed in the following proportions: 1:1, 1:2, 1:3, 2:1, and 3:1. The experiments demonstrated that the voltammograms processed using the validation approach had changed. The impact of the sweep rate, the solvent volume, and the pH on the optimal potential fluctuation range for different current sensitivity was investigated. The mutual interferences of chemicals in mixtures and electroactive substances in pharmaceutical dosage forms, particularly effervescent dosage forms, were also investigated. The same combinations were investigated using the direct spectrophotometric approach, which indicated a significant number of spectrum interferences. An adequate separation or an indirect spectrophotometric approach (the apparent content curves method) were utilised to tackle this problem. The developed spectrophotometric and voltammetric techniques were utilised to determine the concentrations of ascorbic acid and acetaminophen in various dose forms (vials, tablets, suppositories and effervescent dosage forms). The outcomes were compared to those obtained by other techniques.

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