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International Journal Of Pharma Professional's Research

Review Article

RECENT ADVANCED - SMARTER TECHNIQUES OF ANALYSIS

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ISSN NO:0976-6723

Abstract

Pharmaceutical analysis has adopted many faster and smarter processes to deals with increasing commercial and social pressure to develop new medicines with reduced costs and improved productivity. These all techniques are nondestructive, rapid, and accurate methods for drugs and dosage forms.

A number of new techniques are discussed like NIR, Direct Analysis in Real Time (DART), Deep Raman spectroscopy include probing of the active ingredients in pharmaceutical capsules in a fluorescent shell, study of counterfeit tablets in glass bottle, the detection of powders in opaque plastic containers, and non-invasive detection of herbs in bottle. The techniques are also used for analysis of crude drugs for its active constituents. Ion mobility spectrometry (IMS) is used to separate and identify ionized molecules in the gas phase based on their ion mobility in a carrier buffer gas. A best method for detection of narcotics, abused drugs and other harmful substances in trace quantity also. The other technique Ultra high performance liquid chromatography (UPLC) has advantage of reduced run time, less solvent consumption and increased peak capacities. It used for the quality evaluation of herbs and medicines. DOSY NMR technique gives information of active as well as excipients in form of multivarient fingerprint. Tera hertz pulse technique techniques uses electromagnetic spectrum between microwaves and infrared.

Keywords: - NIR, Direct Analysis in Real Time, Raman Spectroscopy, IMS, DOSY.

Introduction

Ensuring quality of medicine is becoming more important and challenging. This generates the need for more fast and smart technique to fulfill the requirement. New techniques that can improve the efficiency of pharmaceutical analysis are being developed. Some of the techniques are discussed in the review. [1]

Near Infra Red Spectroscopy (NIR)

The NIR method relies on the spectra-structure correlations existing between a measured spectral responses caused by harmonics of the fundamental vibrations occurring at infrared frequencies. The response of the near infrared spectrometer is proportional to changes in the concentration of chemical components, or in the physical characteristics (scattering/absorptive properties) of samples undergoing analysis. The technique is used for determination of identity of various organic drugs and bio materials, to determine active constituent in the drug and is non-invasive technique. The spectra range used is 780-2500nm and is a robust technique for industrial use. The penetration depth is more and there are many modes of measuring. It gives powerful multivariate results. In all, it is fast technique with simple sample preparation and a non-destructive technique for online process applications. [1]

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NIR-CI (Near Infrared Chemical Imaging)[2]

NIR-CI uses a two-dimensional detector array, in contrast to mapping techniques that employ single-point or linear detectors. By eliminating the need to move the sample relative to the detector (the step and acquire mode still used

by mapping instruments), the data collection process is accelerated, and there are no moving parts. This is an attractive feature for at- or near-line applications. The large format array, easily configurable sample illumination, and excellent signal-to-noise characteristics, result in image fidelity that is superior to any other vibrational imaging technique. Data acquisition speeds are high, and it is possible to collect 81,920 complete NIR spectra in <2 minutes, capturing images over sample areas as large as 40 x 32 mm. For routine measurements, where only a few wavelengths are required, this time can be reduced to a few seconds for almost real-time imaging. [3] There are no compromises between spectral content and image quality. Data analysis routines can be automated for push-button performance.

Applications: NIR-CI can solve problems for a wide range of sample types and applications, ranging from pharmaceuticals tablets and granules, to grains and seeds, to household products such as dish washing blocks. By simply changing an objective, a standard laboratory system can be quickly configured for microscopic imaging or macroscopic imaging. [4]

1).During product development to optimize performance, manufacturability, and reproducibility

2).Through process development to eliminate costly scale-up problems before they occur.

3).As a QA/QC tool to verify conformity to physical and chemical specifications.

4).For manufacturing root cause analysis.

5).As a general research and development tool to explore structure/function relationships.

6).For high-throughput, high-speed chemical ID screening.

Freedom from sample preparation high quality spectra can be acquired from oddly shaped rough and highly colored samples. Also, multiple samples that are not organized or arranged in a matrix can be analyzed. NIR-CI retains many of the attributes that make traditional NIR single-point spectroscopy an industrial work-horse, such as minimal sample preparation, flexible sample presentation, robust and easy to use instrumentation, and rapid data acquisition. The NIR-CI systems are built on a rugged foundation of solid state components there are no moving parts there are no moving parts to wear out or become misaligned. Operation outside of a controlled laboratory environment is no problem. The NIR-CI systems are the only comprehensive chemical imaging technology with complete product continuity from the lab to the process floor. The technology and its implementation are the same whether the system is performing cutting edge research or routine QA/QC monitoring, eliminating the costs associated with new method development for changing deployments.

NIR-CI as a QA/QC tool [5]

By providing robust, reliable and automated data collection capabilities, coupled tightly with quantitative, statistical, objective, reproducible and automated data processing tools, NIR chemical imaging has moved rapidly from a specialized technology into a routine and highly desirable quantitative analytical tool. Robust instrumentation, modular software that enables the development of turnkey solutions, and instrument platforms that transition from R&D to process environments are just some of the attributes that contribute to the usefulness of NIR chemical imaging as a routine QA/QC tool. Example is shown in figure 1. [6]

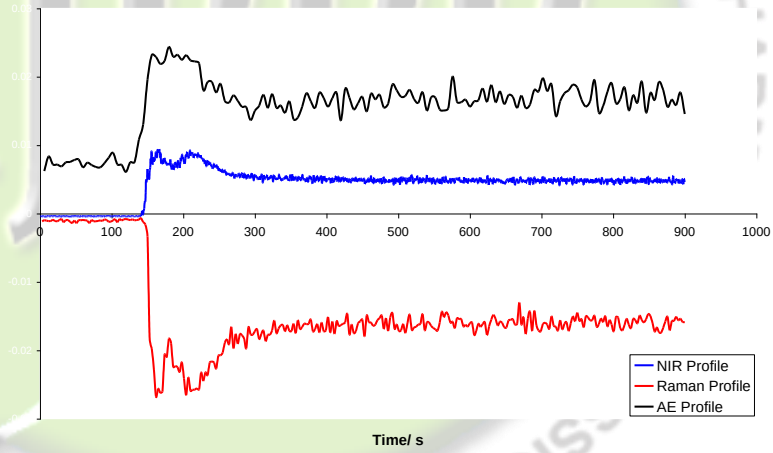

Fig.1.

Applications

1).Monitoring blending for completeness

2).Ensuring content uniformity[7]

3).Assessing coating thickness and uniformity [8]

4).Visualization of chemical composition in granules

5).Characterize particle size and distribution of components in finished products

6).Identifying polymorphs

7).Screening and identification of contaminants

8).Evaluating coating uniformity and thickness

9).Chemical gradients in household materials

10).Defect sites in polymers [9]..

11).Contaminants in biological samples.

12).Structure/function relationships

Deep Raman spectroscopy [10]

Previously, it was used as surface or near surface technique now due to advances it is used as deep spectroscopy technique. Example of use of the technique include probing of the active ingredients in pharmaceutical capsules despite a fluorescent shell, study of counterfied tablets in glass bottle, the detection of powders in opaque plastic containers, and non-invasive detection of coca. Raman spectra can be obtained from bulk solids, liquids, tablets[11], polymers, [12] paper, etc. with little or no sample preparation. Analysis can also be carried out through many containers such as glass bottles, Pyrex® reaction vessels, plastic containers, blister packs, bags, etc. [13]

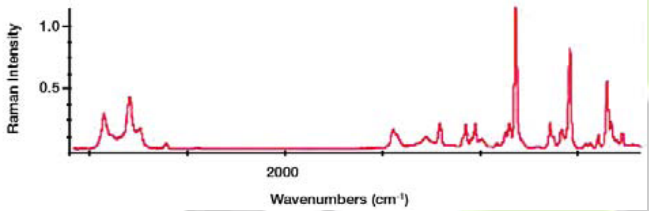

Fig 2. Sample Raman spectrum of a mixture.[14] N

In Raman imaging, the spectra are taken over a predetermined sample surface area in a set sequence and with a defined point interval (spatial resolution). This set of data is displayed as an image where areas of high Raman intensity are shown in one color (normally red) and areas of lower intensity are shown in a different color (normally blue). This variation in color may simply reflect a difference in concentration of the same material across the sample, or may show variation in chemical composition. The Spectrum software allows the user to display these individual spectra and generate chemical distribution images of the components in the sample. A visible image survey of the sample is acquired using the built- in video camera and motorized stage. [15] The area where the Raman image is acquired is comparable with this visible image as shown in figure 3.

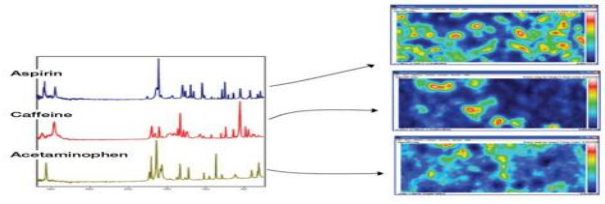

Fig.3 Chemical distribution images of components in a sample

Direct Analysis in Real Time (DART) [16]

DART is a no-preparation sampling technique for MS and works like a solid probe at atmospheric pressure. It operates in open air with no exposed high voltages. The DART design is based on the reactions of electronic or vibronic excited state species with reagent molecules and polar or nonpolar analytes present near the inlet of mass spectrometer.

1).In the source, gas flows through a discharge chamber where an electric potential initiates an electrical discharge producing ions, electrons, and excited-state species in a plasma.

2).The gas flows into a second chamber where a second biased electrode removes ions from the gas stream.

3).Flowing into a third region, the gas can be heated, if desired, then exits through a third perforated electrode or grid toward mass spectrometer sampling orifice.

DART samples any material held in its ionization stream. It produces instantaneous response and does not need any solvents. Thus DART provides a means for rapid analysis of samples with no solvents or sample preparation. The complete analysis of large numbers of samples from sources ranging in scale from LC fraction collection tubes, to solids, to aerosol sprayed on sampling tips. Direct Analysis in Real Time (DARTtm) offers a simple solution to screening for counterfeit drugs. DART can detect the presence or absence of drugs in medicines within seconds by simply placing the pill or medicine in front of the mass spectrometer. In combination with the AccuTOFTM is an ideal technology for conducting analysis of material in open air, combining the accuracy of time of flight with revolutionary ion source. AccuTOFTM –DARTTM combination provides exact masses and accurate isotopic patterns that provide elemental compositions for known and unknown substances. The samples are placed in front of the DART with no sample preparation and the mass spectra are obtained within seconds.

Applications

1).Direct Analysis in Real Time (DARTtm) is capable of analyzing drugs in pills and capsules with no sample preparation. In most cases, the pill can simply be placed in front of the DART and the active ingredients can be detected within seconds. The examples include prescription drugs, over-the-counter medicines, and illicit drugs that were confiscated by a law-enforcement agency.

2).Instantaneous Detection of the "Date-Rape" Drug – GHB.

3).Detection of active constituents from natural drugs like Lycopene in Tomato Skin, Opiates in Single Poppy Seeds,

Capsaicin in Chili Peppers, sesquiterpenes, gingerols, gingerones and shogaols in ginger rhizome, curcumin in turmeric,[17] by placing drug in front of a DART source.

4).Determination of polymer composition.

Ion mobility spectrometry

Ion Mobility Spectrometry (IMS) is an analytical technique used to separate and identify ionized molecules in the gas phase based on their ion mobility in a carrier buffer gas. Miniaturization of such instruments can potentially provide "shirt pocket" devices that deliver information about ambient chemical species. It is a useful method for detection and determination of narcotics, abused drugs and other harmful substances. Laboratories and production facilities now have an alternative to HPLC that can provide important advantages in speed, sensitivity and robustness. The new method utilizes a technology called ion mobility spectrometry that is similar to mass spectrometry. IMS devices come in a wide range of sizes (often tailored for a specific application) and are capable of operating under a broad range of conditions. Systems operated at higher pressure (i.e. atmospheric conditions, 1 atm or 1013 mbar) are also accompanied by elevated temperature (above 100°C), while lower pressure systems (1-20 mbar) do not require heating.

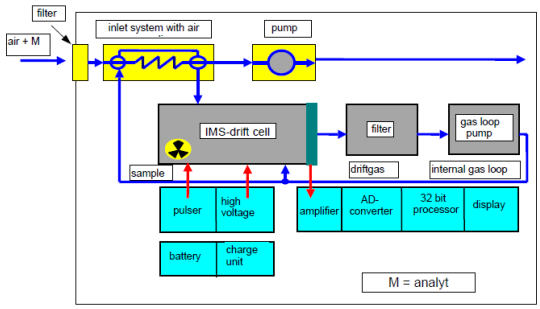

Figure 4 IMS construction, principle and main modules [18]

IMS has the advantage that it can determine the presence or absence of an organic chemical in seconds with virtually no learning curve for the user. Because of its speed, small footprint and low detection limits, thousands of IMS instruments are now in use for detecting explosives, illicit drugs, and chemical warfare agents. More recently, IMS has begun moving into the laboratory based on advantages such as being able to analyze a typical sample in under 30 seconds compared to 30 to 90 minutes for HPLC. The sensitivity of IMS also compares very favorably to HPLC - it can detect quantities from 0.1-10 nanograms of most pharmaceutical compounds. In the pharmaceutical industry, IMS is uses includes

1).Cleaning validations, demonstrating that reaction vessels are sufficiently clean to proceed with the next batch of pharmaceutical product; quality control and chemical monitoring.

2).IMS is also used for analyzing the composition of drugs produced, thereby finding a place in quality assurance and control. [19]

3).As a research tool ion mobility is becoming more widely-used in the analysis of biological materials, specifically, proteomics and metabolomics. For example, IMS-MS using MALDI as the ionization method has helped make advances in proteomics, providing faster high-resolution separations of protein pieces in analysis. [20]

If the application requires quantifying or identifying very low concentrations, IMS is likely the most effective technique.

IMS in Quality Control

The speed and small footprint of IMS instruments means that they can serve as an excellent quality control tool. Any situation where an organic compound is supposed to be present or not present on a product is a viable application candidate. IMS has been used to detect the presence of flavorings, antibiotics, residual allergenics and many other materials. The high speed of IMS means that it can provide HPLC-like analysis at production speeds; compound identification and/or quantification can be accomplished in under 30 seconds. Today's instruments are rugged enough to easily survive in a factory environment. IMS is an analytical technique with high operational speed, favorable ease of use, and exceptionally high sensitivity to a wide range of organic compounds. The method is just beginning to enter the ranks of analytical tools in high-value manufacturing industries. Applications showing great promise are found in pharmaceutical manufacturing, a variety of quality control applications as well as chemical monitoring applications. Utilizing IMS can result in dramatic cost savings from increasing the utilization of costly production equipment, freeing analytical chemist time and rapid analysis time.

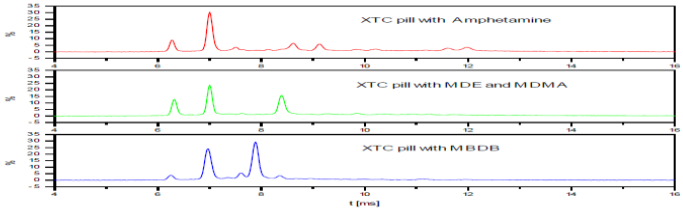

Figure 5 IMS Spectra of street drug pills containing several amphetamines [18]

DOSY NMR

DOSY NMR is a two-dimensional NMR experiment, one dimension accounts for conventional chemical shifts and the other for diffusion coefficients. The use of NMR for measuring the self-diffusion coefficients of molecules in solution is based on a pulsed field gradient (PFG) simulated spin-echo (STE) experiments. The rate of signal decay is directly related to the diffusion coefficient of the molecule, which depends on molecular weight and other hydrodynamic properties like size, shape, charge and on its surrounding environment including temperature, aggregation state etc. [21] Consequently, the signal of each component decays with different diffusion rates as the gradient strength increases, constructing a bilinear NMR data set of a mixture. By calculating the diffusion coefficient for each component, it is possible to obtain a two-dimensional NMR spectrum: one dimension is for the conventional chemical shift and the other for the diffusion coefficient. The most interesting point is that this two-dimensional NMR allows non-invasive "chromatography" to obtain the pure spectrum for each component, providing a possible alternative for LC-NMR that is more expensive and time-consuming. Potential applications of DOSYNMR include identification of the components and impurities in complex mixtures, such as body fluids, or reaction mixtures, and technical or commercial products, e.g. comprising polymers or surfactants. [22-23]

The technique gives information of active as well as excipients in form of multivarient fingerprint. [24] The experiments do not need complicated setups and the method can be easily standardized and automated. The technique is nondestructive nature. The DOSY method allows measuring the translational self-diffusion of molecules in a solution. Based on the analysis of mono- and multi exponential decays, spectra of the mixture components can be separated dependent on their apparent diffusion coefficients. Thus the powerful advantage of the DOSY technique was ignored for a long time.

Ultra High Performance Liquid Chromatography(UPLC)

An ultra-performance liquid chromatography (UPLC) method has been developed and validated for the quality evaluation by various herbal drugs by chemical fingerprinting analysis. It has advantage of reduced run time, less solvent consumption and increased peak capacities. UPLC presents the possibility to extend and expand the utility of conventional HPLC, a widely used separation science. UPLC presents the possibility to extend and expand the utility of conventional HPLC, a widely used separation science. [25]

UPLC refers to Ultra Performance Liquid Chromatography. It improves three areas: chromatographic resolution, speed and sensitivity analysis. It uses fine particles and saves time and reduces solvent consumption. The UPLC is based on the principal of use of stationary phase consisting of particles less than 2 μm (while HPLC columns are typically filled with particles of 3 to 5 μm). The underlying principles of this evolution are governed by the van Deemter equation, which is an empirical formula that describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency).

Advantages

1).Decreases run time and increases sensitivity

2).Provides the selectivity, sensitivity, and dynamic range of LC analysis

3).Maintaining resolution performance.

4).Expands scope of multi residue Methods

5).UPLC's fast resolving power quickly quantifies related and unrelated compounds

6).Faster analysis through the use of a novel separation material of very fine particle size

7).Operation cost is reduced

8).Less solvent consumption

9).Reduces process cycle times, so that more product can be produced with existing resources

10).Increases sample throughput and enables manufacturers to produce more material that consistently meet or exceeds the product specifications, potentially eliminating variability, failed batches, or the need to re-work material

11).Delivers real-time analysis in step with manufacturing processes

12).Assures end-product quality, including final release testing

Disadvantages

Due to increased pressure requires more maintenance and reduces the life of the columns of this type.

So far performance similar or even higher has been demonstrated by using stationary phases of size around 2 μm without the adverse effects of high pressure. In addition, the phases of less than 2 μm are generally non-regenerable and thus have limited use.

Applications

1).Analysis of Natural Products and Traditional Herbal Medicine

2).Study of Metabonomics / Metabolomics

3(.Study of ADME (Absorption, Distribution, Metabolism, Excretion) Screening

UPLC/MS/MS

UPLC/MS/MS operating with rapid, generic gradients has been shown to increase analytical throughput and sensitivity in high throughput pharmacokinetics or bioanalysis studies, including the rapid measurement of potential p450 inhibition, induction, and drug-drug interactions. As well, since this UPLC-based approach can help labs pre-emptively determine candidate toxicity and drug-drug interactions, it enables organizations to be more confident in the viability of candidate medicines that do progress to late-stage clinical trials. Tandem quadrupole MS combines with UPLC in ADME screening for sensitivity and selectivity with fast analyses of samples in matrix to be achieved with minimal cleanup, using MRM (multiple reaction monitoring) for detection and automated compound optimization.

Applications

1).Bioanalysis / Bioequivalence Studies

2).Dissolution Testing

3).Forced Degradation Studies

4(.Manufacturing / QA / QC

Identity, purity, quality, safety and efficacy are the important factors to be considered while manufacturing a drug product .The successful production of quality pharmaceutical products requires that raw materials meet purity specifications. That manufacturing processes proceed as designed. Those final pharmaceutical products meet, and hopefully exceed, defined release specifications. Continued monitoring of material stability is also a component of quality assurance and control.

UPLC is used for the highly regulated, quantitative analyses performed in QA/QC laboratories.The supply of consistent, high quality consumable products plays an important role in a registered analytical method. The need for consistency over the lifetime of a drug product which could be in excess of 30 years is essential in order to avoid method revalidation and associated production delays. [26]

Tera Hertz Pulse Technique [27]

The 'terahertz gap' encompasses frequencies from ~0.3 THz to ~10 THz in the electromagnetic spectrum, lying between microwave and infrared. The radiation (T-Ray) in this band is invisible to the naked eye. Unlike X-ray, terahertz radiation is intrinsically safe, non-destructive and non-invasive. It creates a powerful spectroscopic and imaging technique for characterizing molecular structures. Terahertz spectroscopy enables 3D imaging of structures and materials, and the measurement of the unique spectral fingerprints of different chemical and physical forms. Terahertz radiation has a few remarkable properties. Many common materials and living tissues are semi-transparent and have 'Terahertz fingerprints', permitting them to be imaged, identified, and analyzed. Moreover, the non-ionizing properties of Terahertz radiation are inherently safe for screening application.

Terahertz spectroscopy provides rapid identification of the different crystalline forms of drug molecules – the polymorphs – which can display different solubilities, stabilities and bioavailability and therefore are an important factor in the therapeutic efficacy of a drug. Detecting and identifying the different polymorphs and understanding the mechanism and dynamics of polymorphic inter-conversion, is an important milestone in selecting the optimum form for further development and manufacture. Not only is it possible to detect the differences between pure specimens of the polymorphs but terahertz spectroscopy can distinguish between specific polymorphic forms in the tablet formulation. Terahertz spectroscopy can differentiate between different hydrate forms. Lactose, one of the most commonly used excipients in the pharmaceutical industry, forms at least three different hydrates: the most widely used α-monohydrate, the α-anhydrate and a β-anhydrate form. These three hydrate forms exhibit terahertz spectra that can be used for both quantitative and qualitative analysis. The terahertz region also provides unique sensitivity to lattice structure enabling qualitative and quantitative analysis of crystalline and amorphous materials.

Applications

1). Terahertz imaging gives an unparallel certainty about the integrity of tablet coatings and the matrix performance of tablet cores.

2). Terahertz image can be optimized for performing 3D analysis on tablets.

3). It can enable customers to determine coating integrity and thickness, detect and identify localized chemical/physical structure such as cracks or chemical agglomeration within a core and to interrogate embedded layers (such as an interface between two layers) for delamination and integrity.

4).Terahertz measurements may well become the primary method for the nondestructive determination of coating thickness, requiring little or no calibration for most coatings and substrates.

5).It can reveal the thickness, uniformity, distribution and coverage of simple and complex coatings.

6).Terahertz image can also detect embedded layers and localized chemical/physical structural features in the cores of intact tablets to confirm 3D morphology and blend uniformity.

Conclusion

These techniques like NIR, DART, DOSY NMR, UPLC, Tera hertz pulse techniques and various other techniques are incentives for PAT (Process Analytical Technique). These all techniques are non-invasive, no sample preparation required, fast and accurate. The analytical benefits of these techniques are real-time determination and potential for quantification of components. The quality advantages, are more consistent product and tighter control of specification.These techniques also provide business benefits of increased quality and productivity and fewer 'wasted' batches. All these techniques are non-invasive with advantages of removal of errors associated with manual sampling, reduced risk of contamination and less chances of containment of dangerous materials. The main disadvantages are optical techniques require window and the number of sample points to be decided. The quality evaluation of traditional drugs represents a particular challenge owing to the complexity of the matrix, which renders separation and identification of the individual components extremely difficult but some of these techniques like DART can be used for fingerprinting that play a dominant role in quality control.

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