

Evaluation of Crude Drugs

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Abstract:

Evaluation is a systematic determination of a subject's merit, worth and significance, using criteria governed by a set of standards. It can assist an organization, program, design, project or any other intervention or initiative to assess any aim, realisable concept/proposal, or any alternative, to help in decision-making; or to ascertain the degree of achievement or value in regard to the aim and objectives and results of any such action that has been completed. The primary purpose of evaluation, in addition to gaining insight into prior or existing initiatives, is to enable reflection and assist in the identification of future change.

Keywords: crude drugs, evaluation of crude drugs, organoleptic evaluation etc.

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I. Crude Drugs:

Crude drugs are vegetable or animal drug that contain natural substances that have undergone only the processes of collection and drying. The term natural substances refers to those substances found in nature that have not had man-made changes made in their molecular structure. They are used as medicine for human being and animal, internally and externally for curing disease, e.g., Senna and Cinchona.

A **crude drug** is any naturally occurring, unrefined substance derived from organic or inorganic sources such as plant, animal, bacteria, organs or whole organisms intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in humans or other animals.[1]

II. Evaluation of crude drugs

Organoleptic and morphological evaluation: Evaluation by means of organs of senses knowing the color, odor, taste, size, shape and special features like texture.

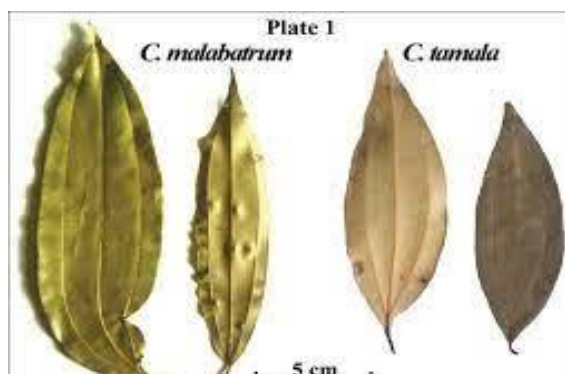
Microscopic: For identification of the pure powdered drug. This method allows more detailed examination of a drug and their identification by their known histological characters. Microscope by the virtue of its property to magnify, permits minute sections under study to enlarge so that leaf constants, stomatal index, palisade ratio can be determined.

Biologic: Pharmacological activities of drugs are evaluated by bioassays. When the estimation of potency of crude drug or its preparations are done by means of measuring its effect on living organisms like bacteria, fungal growth, or animal tissue, it is known as biological effect of the drug, compared to the standard drug. By these methods, a crude drug can be assessed and further clinical trial can be recommended.

Chemical: Chemical assays are best to determine potency and active constituents. It comprises different test and assays. The isolation, purification and identification of active constituents are the methods of evaluation. Quantitative chemical test such as acid value, saponification value etc are also covered under these techniques.

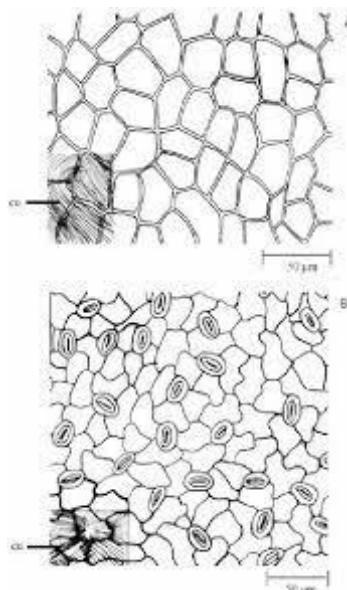
Physical: Physical constants are applied to active principles. These are helpful in evaluation with reference to moisture content, specific gravity, density, optic rotation etc.[2]

ORGANOLEPTIC EVALUATION



- Examination of the drug by color, odor, shape size, taste, touch, texture, and sound is known as Organoleptic evaluation.
- e.g. taste of fennel is sweet. Taste of clove is pungent. Leaf of datura is hairy.
- While evaluating by this method there are certain restrictions like changes in shape & size of drugs during drying & packing so it is Difficult to the study the drug by organoleptic evaluation.
- e.g. length of the cinnamon quill is 1 meter but mostly it is found in small pieces in the market.
- Digitalis leaves crumple in small pieces during drying and packing.

MICROSCOPICAL EVALUATION



- Every species has a unique anatomy, the study of which helps us in their identification process.
- Microscopic evaluation is useful for the organized drugs (i.e. drugs having cellular structure). T. S. & L.S. of the drugs are observed under the microscope.
- T. S. & L.S. of the drugs are studied under the microscope with the help of the staining agent.
- Special attention is given to the type of tissues, their arrangement, presence or absence of special substances like calcium oxalate crystals, starch grains, size and shape of starch grains, cell contents etc.
- e.g. Nux vomica have lignified trichomes, Fennel contains vascular bundles which are surrounded by reticulated parenchyma and shows the presence of vitae which secrete volatile oil.
- **Microchemistry:** Sometimes small quantities of chemical reagent are used on sections to highlight specific cells or structures.
- e.g. If we want to observe starch grain then we have to use dilute iodine solution the area of T. S. containing starch grains becomes blue due to iodine.

- To locate strychnine & Brucine in Nux- vomica seeds, we use ammonium vanadate. Thus the use of small quantities of drugs & chemical reagents in microscopy is known as microchemistry.
- Following are the examples of microscopic evaluation. (Leaf constants)
- **A) Stomatal No. :** - It is an average no. of stomata present in 1 sq. mm of the epidermis.
- The total no is constant for a given drug. e.g Drug Stomatal no. *Datura stramonium* 87, *D.innoxia* 141.
- **B) Stomatal Index:** - It is the percentage which the number of stomata forms to the total no. of epidermal cells, each stoma being counted as one cell.

Microscopic evaluation is indispensable in the initial identification of herbs, as well as in identifying small fragments of crude or powdered herbs, and in the detection of adulterants (e.g. insects, animal faeces, mold, fungi, etc.) as well as identifying the plant by characteristic tissue features. Every plant possesses a characteristic tissue structure, which can be demonstrated through study of tissue arrangement, cell walls, and configuration when properly mounted in stains, reagents, and media. Lignin stains red or pink with a drop of phloroglucinol and concentrated hydrochloric acid. Mucilage is stained pink with ruthenium red, and N/50 iodine solution stains starch and hemicellulose blue.

The characteristic features of cell walls, cell contents, starch grains, calcium oxalate crystals, trichomes, fibres, vessels, etc. have been studied in details. Surinam quassia is recognized by the absence of calcium oxalate and presence of uniseriate medullary rays, crystal fibres, and wavy medullary rays of cascara bark, lignified trichomes, and plasmodesma in nux vomica. Stone cells are absent in the frangula bark, whereas they are present in cascara. Presence of pith in rhizomes and absence in roots, warty trichomes of senna, and presence or absence of crystals of aloin indicates different varieties of aloes, glandular trichomes of mint, etc. The powder of clove stalks contains sclereids and calcium oxalate crystals, but cloves do not contain these two.

Linear measurements include size of starch grains, length and width of fibres, trichomes, etc. The diameter of starch grains present in ipecacuanha assists in distinguishing its varieties. The diameter of starch grains in cassia bark distinguishes from cinnamon and detects senna stalk in powdered senna leaf. The size of the stomata in leaves of *Barosmabetulina* distinguishes it from other species of Barosma. The diameter of phloem fibres aids the detection of cassia in cinnamon, and the width of the vessel helps to detect clove stalks in powdered cloves. Measurements of diameter for the identification of commercial starches and for the detection in them of foreign starch are few examples of linear measurements.

Determination of leaf constants include: stomatal number, stomatal index, vein islet, vein termination number, and palisade ratios. Stomatal number is average number of stomata per sq. mm of epidermis of the leaf.

Stomatal index: It is the percentage which the numbers of stomata form to the total number of epidermal cells, each stoma being counted as one cell. Stomatal index can be calculated by using the following formula:

$$\text{Stomatal Index (S.I.)} = \frac{S}{E + S} \times 100$$

where,

S = number of stomata per unit area and

E = number of epidermal cells in the same unit area.

Timmerman (1927) and Rowson (1943) were amongst the first few to investigate leaf drugs for stomatal number and stomatal index.

Vein-islet number: It is defined as the number of vein islets per sq. mm of the leaf surface midway between the midrib and the margin. It is a constant for a given species of the plant and is used as a characteristic for the identification of the allied species. Levin in 1929 determined vein-islet numbers of several dicot leaves.

Veinlet termination number: It is defined as the number of veinlet termination per sq. mm of the leaf surface midway between midrib and margin. A vein termination is the ultimate free termination of veinlet. Hall and Melville in 1951 determined veinlet termination number of distinguishing between Indian and Alexandrian Senna.

Palisade ratio: It is defined as the average number of palisade cells beneath each epidermal cell. Unlike vein-islet number for the determination of which an unbroken portion of the leaf is required, palisade ratio can be determined with the powdered drug. The technique of palisade ratio determination was introduced by Zorning and Weiss (1925) in their studies on Compositae.

One example is vein-islet number of Alexandrian senna is 25–29.5, whereas Indian senna is 19.5–22.5. Stomatal index of Alexandrian senna is 10–15, whereas that of Indian Senna is 14–20.



Quantitative Microscopy (Lycopodium Spore Method)

This is an important technique employed in identification of crude drug when chemical and physical methods are inapplicable. Using this, one can determine the proportions of the substances present by means of the microscope, using the Lycopodium spore method.

The powdered drugs with well-defined particles which may be counted—for example, starch grains or single-layered cells or tissues—the area of which may be traced under suitable magnification or the objects of uniform thickness, and the length of which, can be measured under suitable magnification and actual area calculated are usually evaluated using this method.

Adulterated starchy drugs can be determined by counting the number of starch grains per mg and calculating the amount from the known number of starch grains per mg of the pure starch or starchy material.

Thus, if spent ginger is the adulterant, one knows that ginger contains 286,000 starch grains per mg, and the amount used as an adulterant can be calculated by using this figure. The percentage purity of an authentic powdered ginger is calculated using the following equation:

$$[N \times W \times 94,000 \times 100] / [S \times M \times P] = \% \text{ purity of drugs}$$

where,

N = number of characteristic structures (e.g. starch grains) in 25 fields;

W = weight in mg of lycopodium taken;

S = number of lycopodium spores in the same 25 fields;

M = weight in mg of the sample, calculated on basis of sample dried at 105°C; and

P = 2,86,000 in case of ginger starch grains powder.

If the material is one for which a constant is not available, it is necessary to determine one by a preliminary experiment.

CHEMICAL EVALUATION

The chemical evaluation includes qualitative chemical tests, quantitative chemical tests, chemical assays, and instrumental analysis. The isolation, purification, and identification of active constituents are chemical methods of evaluation. Qualitative chemical tests include identification tests for various phytoconstituents like alkaloids, glycosides, tannins, etc. The procedures for the identification tests of various phytoconstituents are given under their respective chapters in the text, where it could be referred. Examples of identification of constituents are: copper acetate used in the detection of colophony present as an adulterant for

resins, balsams, and waxes; Holphen's test for cottonseed oil and Baudouin's test for sesame oil in olive oil; the test with acetic and nitric acids for Gurjun balsam in copaiba; Van Urk's reagent for ergot; Vitali's morins reaction for tropane alkaloids; iodine for starch; murexide test for purine bases, etc. are examples of this evaluation.

Quantitative chemical tests such as acid value (resins, balsams), saponification value (balsams), ester value (balsams, volatile oils), acetyl value (volatile oils), etc. are also useful in evaluation of a drug by means of chemical treatment.

Chemical assays include assays for alkaloid, resin, volatile oil, glycoside, vitamins, or other constituent. Few examples are the assay of total alkaloid in belladonna herb, the total alkaloid and nonphenolic alkaloid in ipecacuanha, the alkaloid strychnine in nux vomica, the resin in jalap, and the vitamins in cod-liver oil. The results obtained can conclude the presence of inferior or exhausted drug and, by proving absence of the assayed constituent, it will suggest complete substitution of a worthless article.

Instrumental analyses are used to analyse the chemical groups of phytoconstituents using chromatographic and spectroscopic methods. Chromatographic methods include paper chromatography, thin-layer chromatography, gas chromatography, high-performance liquid chromatography, and high-performance thin-layer chromatography. Spectroscopic methods include ultraviolet and visible spectroscopy, infrared spectroscopy, mass spectroscopy, and nuclear magnetic spectroscopy.[3]

PHYSICAL EVALUATION

In crude plant evaluation, physical methods are often used to determine the solubility, specific gravity, optical rotation, viscosity, refractive index, melting point, water content, degree of fibre elasticity, and other physical characteristics of the herb material.

Solubility

Drugs specific behaviours towards solvents are taken into consideration. This is useful for the examination of many oils, oleoresins, etc. Few examples are the solubility of colophony in light petroleum, the solubility of balsam of Peru in solution of chloral hydrate, the solubility of castor oil in half its volume of light petroleum and the turbidity produced with two volumes of the solvent; the solubility of balsam of Peru in an equal volume of alcohol, 90%, and the production of a turbidity with a larger volume; castor oil is soluble only in three volumes of 90% alcohol, while the adulterated form it shows good solubility in alcohol. Alkaloidal bases are soluble in organic solvents and alkaloidal salts are soluble in polar solvents.

Optical Rotation

Anisotropic crystalline solids and samples containing an excess of one enantiomer of a chiral molecule can rotate the orientation of plane-polarized light. Such substances are said to be optically active, and this property is known as optical rotation. The enantiomer that rotates light to the right, or clockwise when viewing in the direction of light propagation, is called the dextrorotatory (d) or (+) enantiomer, and the enantiomer that rotates light to the left, or counterclockwise, is called the levorotatory (l) or (−) enantiomer. Few examples of drugs with this property are eucalyptus oil (0° to +10°), honey (+3° to {15°), Che-nopodium oil ({30° to {80°), etc.

Refractive Index

Refractive index is defined as the property of a material that changes the speed of light, computed as the ratio of the speed of light in a vacuum to the speed of light through the material. When light travels at an angle between two different materials, their refractive indices determine the angle of transmission refraction of the light beam. In general, the refractive index varies based on the frequency of the light as well; thus, different colours of light travel at different speeds. High intensities can also change the refractive index. This could be used as a parameter in evaluating the herbal drugs; for example castor oil 1.4758 to 1.527, clove oil 1.527 to 1.535, etc.

Specific Gravity

It is also known as relative density. The ratio of the mass of a solid or liquid to the mass of an equal volume of distilled water at 4°C (39°F) or of a gas to an equal volume of air or hydrogen under prescribed conditions of temperature and pressure. Some examples of specific gravity of drugs are cottonseed oil 0.88–0.93, coconut oil 0.925, castor oil 0.95, etc.

Viscosity

Viscosity is the resistance of a fluid to flow. This resistance acts against the motion of any solid object through the fluid and also against motion of the fluid itself past stationary obstacles. Viscosity of a liquid is constant at a given temperature and is an index of its composition. Viscosity also acts internally on the fluid between slower- and faster-moving adjacent layers. Since it is constant at a given temperature, it is used as an evaluation parameter; for example, pyroxylin kinematic viscosity, 1100–2450 centistokes.

Melting Point

The melting point of a solid is the temperature at which it changes state from solid to liquid. Plant constituents have very sharp and constant melting points. As far as crude drugs are concerned, melting point range has been fixed due to mixed chemicals. The following drugs could be evaluated using this parameter; for example, beeswax 62–65°C, wool fat 34–44°C, agar melts at 85°C, etc.

Moisture Content

The moisture content of a drug will be responsible for decomposition of crude drugs either producing chemical change or microbial growth. So the moisture content of a drug should be determined and controlled. The moisture content is determined by heating a drug at 105°C in an oven to a constant weight. Following are the examples of two crude drugs with their moisture content limit: the moisture content of *Digitalis* and *Ergot* should not be more than 5% w/w and 8% w/w, respectively.

Ultraviolet Light

Certain drugs fluoresce when the cut surface or the powder is exposed to ultraviolet radiation, and it is useful in the identification of those drugs. Some pieces of rhapontic, Indian, and Chinese rhubarb are very difficult to distinguish, and it is very difficult in powdered form, but examination in ultraviolet light gives such marked differences in fluorescence that the varieties can be easily distinguished from each other.[4]

Ash Values

The determination of ash is useful for detecting low-grade products, exhausted drugs, and excess of sandy or earthy matter. Different types of ash values are used in detection of crude drugs like, total ash, acid-insoluble ash, water-soluble ash, and sulphated ash.

Total ash is useful in detecting the crude drugs that are mixed with various mineral substances like sand, soil, calcium oxalate, chalk powder, or other drugs with different inorganic contents to improve their appearance, as is done with nutmegs and ginger. The maximum temperature used for total ash should be not more than 450°C because alkali chlorides that may be volatile in higher temperatures would be lost.

Acid-insoluble ash means the ash insoluble in dilute hydrochloric acid. It is often of more value than the total ash. The majority of crude drugs contain calcium oxalate, and the quantity of calcium oxalate varies very frequently. So total ash of a crude drug varies within wide limits for specimens of genuine drug, for example, rhubarb, total ash range from 8 to 40%. In this case, the total ash is useless to detect earthy matter adherent to such a drug. So acid-insoluble ash would be preferable for rhubarb. The calcium oxide or carbonate, yielded by the incinerated oxalate, will be soluble in hydrochloric acid when the ash is treated with hydrochloric acid; the remaining ash is weighed, which is known as the acid-insoluble ash. By this we can detect the presence of excessive earthy matter, which is likely to occur with roots and rhizomes and with leaves which are densely pubescent, like those of foxglove, clothed with abundant trichomes secreting resin, as in henbane, and tend to retain earth matter splashed on to them during heavy rainstorms.

The water-soluble ash is used to detect the presence of material exhausted by water. Sulphated ash is done by addition of sulphuric acid in order to get sulphate salts, and the percentage ash is calculated with reference to the air-dried drug. The temperature used for this is above 600°C. The total ash and acid-insoluble ash values of *Guduchi* are not more than 16 and 3%, respectively. The total ash value and water-soluble ash values of ginger are 6 and 1.7%, respectively.

Extractive Values

The extracts obtained by exhausting crude drugs with different solvents are approximate measures of their chemical constituents. Various solvents are used according to the type of the constituents to be analysed. Water-soluble extractive is used for crude drugs containing water-soluble constituents like glycosides, tannins, mucilage, etc.; alcohol-soluble extractive is used for crude drugs containing tannins, glycosides, resins, etc.; and ether-soluble extractives are used for drugs containing volatile constituents and fats.

Extractive Values of Some Crude Drugs

Water-soluble extractive (% w/w)		Alcohol-soluble extractive (% w/w)		Ether-soluble extractives (% w/w)	
aloe	Not less than 25.0	aloe	Not less than 10.0	linseed	not less than 25.0
glycyrrhiza	Not less than 20.0	asafoetida	Not less than 50.0	capsicum	not less than 12.0

Foreign Organic Matters

The parts of the organ or organs other than those parts of drugs mentioned in the definition and description of the drug are known as foreign organic matters. They may be insect, moulds, earthy material, animal excreta, etc. Each and every vegetable drug has their own limits. Few examples of such limits are: garlic should not contain more than 2%, saffron should not contain more than 2%, satavari should not contain more than 1%, etc.[5]

BIOLOGICAL EVALUATION

The plant or extract can then be evaluated by various biological methods to determine pharmacological activity, potency, and toxicity. The biological evaluation would serve better than the physical and chemical evaluation for drugs that could not be satisfactorily assayed by these last two methods. Moreover, this is an important method, the crude drugs are considered important only because of their biological effects and this evaluation would conclude the effect. These methods are considered to be less precise, more time-consuming and more expensive. Bioassays should be as simple as possible, and attempts should be made to have access to a large number of different tests so that many biological properties can be screened. The bioassay methods are of three types they are, toxic, symptomatic and tissue or organ methods. Different animals are used in toxic and symptomatic method and isolated organ or tissue is used in the third method.

These assays are conducted by determining the amount of drug of known potency required to produce a definite effect on suitable test animals or organs under standard conditions. Reference standard are used in certain bioassay procedures to minimize errors.

Toxicity studies are performed in suitable animal models to decide the lethal dose and effective dose of crude drugs. Mice are used to test the effects of various vaccines.

Oxytocic activity of vasopressin injection is tested on guinea pigs, and oxytocic injection is assayed on young domestic chickens by injecting into an exposed crural or brachial vein and noticing the changes in blood pressure. Pigeons are used to assay Digitalis glycosides by transfusing the drug through the alar vein to the blood stream and observing the lethal effects. Depressor activities and mydriatic effects of certain drugs are tested in cats and cat's eye, respectively. Anthelmintic drugs are evaluated on worms.

The drugs that have an effect in eyes are assayed on rabbit's eyes. Dogs are used to assay the drugs that exhibit cardiac and gastrointestinal activities. Effects of Ergot are carried out on cock's comb or rabbit's intestine or its uterus. Next to the animals, the studies are carried out in human beings also. In some instances, the effects that are observed from animal studies would be different when tested in humans. The tested biological activities include hepatoprotective activity, hypoglycaemic activity, antiinflammatory activity, antiulcer activity, immunomodulatory activity, etc.

Microbiological assays are carried out to determine the effects of drug in various microorganisms, and this is employed in the identification of antimicrobial drugs. The methods used in this type of assays are agar well-diffusion method, disc-diffusion method, and turbidimetric method. In other microbiological methods, the living bacteria yeast moulds are used for assaying vitamins.

QUANTITATIVE MICROSCOPY

A microscopy technique comprised of light microscopy coupled with modern microscope optics and use of algorithms for the restoration, segmentation, and analysis of digital images for the quantitative analysis of specimens in order to achieve the accurate measurement of "analog" quantities of a digital representation of an image.

Biological Screening of Herbal Drugs

ACACIA GUM

Synonyms

Acacia gum, Acacia vera, Egyptian thorn, Gummi africanum, Gum Senegal, Gummaemimosae, Kher, Sudan gum arabic, Somali gum, Yellow thorn, Indian Gum and Gum Arabic.

Biological Source

According to the USP, acacia is the dried gummy exudation obtained from the stems and branches of *Acacia senegal* (L.) Willd or other African species of Acacia. In India, it is found as dried gummy exudation obtained from the stems and branches of *Acacia arabica* Willd, belonging to family Leguminosae

Geographical Source

Acacia senegal is the characteristic species in the drier parts of Anglo-Egyptian Sudan and the northern Sahara, and is to be found throughout the vast area from Senegal to the Red Sea and to eastern India. It extends southwards to northern Nigeria, Uganda, Kenya, Tanzania and southern Africa. The plant is extensively found in Arabia, Kordofan (North-East Africa), Sri Lanka and Morocco. In India it is found chiefly in Punjab, Rajasthan and Western Ghats. Sudan is the major producer of this gum and caters for about 85% of the world supply.[6]

Cultivation and Collection

Acacia is a thorny tree up to 6 m in height. In Sudan, gum is tapped from specially cultivated trees while in Senegambia, because of extremes of climate; cracks are produced on the tree and the gum exudes and is collected from the wild plants. Acacia trees can be cultivated by sowing the seeds in the poor, exhausted soil containing no minerals. The trees also grow as such by seed-dispersal.

Gum is collected by natives from 6 to 8 years old trees, twice a year in dry weather in November or in February—March. Natives cut the lower thorny branches to facilitate the working and by means of an axe make 2–3 ft long and 2–3 inches broad incision on the stem and branches, loosen the bark by axe and remove it, taking care not to injure the cambium and xylem. Usually they leave a thin layer of bark on xylem. If xylem is exposed, white ant enters the plant and gum is not produced. After injury in winter gum exudes after 6–8 weeks while in summer after 3–4 weeks. It is believed that bacteria finding their way through the incision are more active in summer and gum is produced quickly. The exuded gum is scraped off, collected in leather bags and then is cleaned by separating debris of bark and wood and separating sand, etc., by sieving.[7]

Gum is dried in the sun by keeping it in trays in thin layers for about 3 weeks when bleaching takes place and it becomes whiter. This result in uneven contraction and cracks and fissures are formed on its outer surface and as a result original transparent gum becomes opaque. This process is called ripening of the gum.

III. CONCLUSION

Because substance abuse and delinquency are inextricably interrelated, identifying substance-abusing youth in the juvenile justice system is an important first step for intervening in both their substance abuse and their delinquent behavior. Drug identification strategies, followed by effective interventions, help prevent further illicit drug use and delinquency. Drug testing can be a constructive means of helping youth overcome denial of their substance abuse. As a part of intervention, drug testing can be used to help youth achieve and maintain recovery and curtail other deviant behaviors. Over time, effective drug identification will help juvenile justice agencies achieve the goals of a balanced approach including community protection, youth accountability, and competency development.

Five sites engaged primarily in juvenile probation and three juvenile detention centers implemented the drug identification programs reported in this Summary. Each received assistance from the APPA or the ACA/IBH to establish a drug-testing and intervention program meeting standards based on national research on drug-testing programs. Across the eight demonstration sites, the percentage of positive drug test results obtained from youth ranged from 10 percent in one site to 37 percent in another, a finding that corresponds to other data that show a significant amount of illicit drug use among youth in the juvenile justice system. The most frequent positive results in all sites were for marijuana. In most of the sites, the next highest rate of positive results was for cocaine. However, in all but one site, the percentage of positive results for cocaine was dramatically lower than the percentage of positive results for marijuana. Two sites had several positive tests for PCP

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